The use of seagrass (*Zostera muelleri*) habitat types by seagrass communities in New Zealand.

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Abstract

Seagrasses are a unique marine ecosystem that form extensive beds throughout coastal, estuarine areas and harbour rich and diverse taxonomic groups. Throughout the globe, seagrasses have experienced decline and there is a growing need to understand how patterns in biodiversity and distribution respond to increasing stressor. In New Zealand, seagrass ecosystems consist of one species (*Zostera mulleri*), however, the biodiversity value of these habitats remains understudied. To asses spatial and temporal variation, seagrass associated faunal communities were sampled in 9 locations across New Zealand. One of these locations (Duvauchelle bay) was sampled over the span the year to gain understanding how *Z. muelleri* systems may vary naturally over the seasons. This study exemplifies the highly variable nature of seagrass systems as regions and sites differed in overall species composition and abundance. Southern sites were found to support the most diverse communities, whereas, northern sites supported the most abundant communities. Results suggest that sites that are either in close proximity of large-scale development will experience reductions in their taxonomic communities. Furthermore, surrounding landscape and topography were attributed to changes seen across sites. Only isolated sites supported populations of pipefish, suggesting that pipefish may be useful ecological indicators for *Z. muelleri* systems in New Zealand

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General Introduction

Seagrass is a dominant and productive biogenic habitat in many coastal waters (Spalding *et al.*2003). These marine angiosperms represent the only order of flowering marine plants and possess a variety of morphological adaptations, specialised for the fluctuating brackish conditions of estuaries (Spalding *et al.*2003, Turner and Schwarz 2006). Meadows are conventionally distributed along intertidal and shallow subtidal zones throughout temperate, tropical, and subarctic coastal waters where light availability is high. With a depth range of 2-12m large portions of seagrass beds experience periods of exposure to fluctuations in light and temperature intensities during the low tide, although, in tropical regions where water clarity is high, beds exist as deep as 70m (Dennison *et al.*1993, Morrison *et al.*2014, Spalding *et al.*2003, Turner and Schwarz 2006). While seagrasses can grow on a variety of substrata such as mud, sand, bedrock and rocky reef, extensive beds generally grow in soft substrate that is sheltered from high wave and tidal action (Hemminga and Duarte 2000, Green and Short 2003). Seagrasses can reproduce both sexually and asexually (vegetatively) but growth of beds is maintained primarily through vegetative propagation. Rhizomes create a branching network beneath the substrata that continually grows while producing lateral grass shoots (Hemminga

and Duarte 2000, Turner and Schwarz 2006). Vegetative growth can span over several square kilometres or as an assortment of patches surrounded by unvegetated sediment (Hemminga and Duarte 2000, Turner and Schwarz 2006). Sexual reproduction may not be vital for maintenance, but it has been suggested to be important for long term population dynamics such as re-establishment, connectivity, growth and reproduction (Turner and Schwarz 2006, Morrison *et al.*2014).

Despite receiving less public attention when compared to other biogenic habitats such as coral reefs, seagrasses are among the most productive ecosystems in the world (Constanza *et al.* 1997, Morrison *et al.*2014). In many estuaries, seagrasses act as a multifunctional biogenic habitat, providing vital services that greatly benefit communities within and in adjacent ecosystems (Morrison *et al.*2014). Such services include nutrient cycling, sediment trapping and stabilising, attenuating water flow, carbon sequestration, supporting numerous food webs (detrital, grazing, marine birds ect), habitat provision and refugia from predation (Garcia and Duarte 2001, Hailes 2006, Matheson and Wadhwa 2012, Morrison *et al.*2014, Turner and Schwarz 2006, Widdows *et al.*2008). Leaves and an extensive rhizome system provides a variety of microhabitats that greatly increases both vertical and horizontal heterogeneity. Addition of structural complexity in an otherwise homogenous landscape such as soft sediment or mud has a significant influence on the abundance, diversity and distribution of associated flora and fauna (Bologna and Heck 2000, Spalding *et al.* 2003, Turner and Schwarz 2006). Leaf blades also modify local hydrodynamic conditions by reducing wave and current force, which facilitates the stabilisation of suspended sediments and settlement of organic matter, thus, increases food availability for benthic fauna (Peterson *et al.*2004, Terrados and Duarte 2000).

The provision of refugia is particularly vital for a variety of functional groups that rely on seagrass as nursery habitat (Grech *et al.* 2012, Orth *et al.* 2006, Orth and Van Montfrans 1987). A number of fish species rely on seagrass as nursery habitat for refuge and food during a highly vulnerable life stage. Extensive studies have found that seagrass habitat has notably high densities of juvenile fish species when compared to unvegetated habitat (Gillanders 2006, Heck *et al.*2003, Hemminga and Duarte 2000). Consequently, by increasing survival and growth rates of juvenile fish means seagrasses may likely have influence on population dynamics of fish species out to sea (Bertelli and Unsworth 2014, Dorenbosch *et al.*2004, Verweij *et al.*2008). By providing shelter for prey species, seagrass has the ability to alter predator-prey relationships while simultaneously supporting other trophic levels such as sea turtles, dugongs, tiger sharks and marine birds (Aragones 1999, Heithaus *et al.*2002, Bertelli and Unsworth *et al.* 2014, Turner and Schwarz 2006, Williams and Heck 2000). The concept of seagrass as nursey habitat has gained traction over the past decade and prompted studies from across the globe to determine their relative importance of to fish species that utilise this habitat as juveniles (Beck *et al.*2001, Heck *et al.*2003). Unfortunately, studies for this topic follow a large geographical bias as most are conducted either in North America or Australia.

In New Zealand, seagrass habitats only consist of one species, whereas, seagrass meadows elsewhere routinely consist of multiple coexisting species (Short *et al.* 2007, Turner and Schwars 2006). *Zostera muelleri* is indigenous to New Zealand and parts of southern Australia and represents New Zealand's only species of seagrass (Anderson et al, 2019, Morrison *et al.*2014). Key environmental drivers for survival of this species include photosynthetic parameters such as light availability, nutrients, and water clarity as well as abiotic parameters such as temperature, salinity, hydrodynamic activity, and substrate characteristics (Hemminga and Duarte 2000, Turner and Schwarz *et al.*2006). In New Zealand, *Z. muelleri* grows in abundance along intertidal mudflats of sheltered harbours and estuaries where light availability is high as beds become exposed during low tide. Beds have also been recorded on rocky reef platforms, coastal beaches and in subtidal waters near offshore islands where they remain permanently submerged (Morrison *et al.*2014, Turner and Schwarz 2006). Historical ranges of seagrass in New Zealand remain largely unknown due to a paucity of documentation which makes it difficult to accurately extrapolate the extent of spatial change and degradation over the past century. Nevertheless, there is substantial evidence that seagrasses in New Zealand have experienced great declines in range and condition because of human activity (Orth *et al.*2006, Morrison *et al.*2014, Waycott *et al.*2009). For example, in the eastern Bay of Islands, up to 90% of subtidal seagrass beds were lost between 1961-2006, due to increased sedimentation and nutrient inputs from anthropogenic sources. While subtidal seagrass habitat was once as widespread as intertidal habitat towards the end of the 19th century, permanently submerged beds are now mainly restricted to offshore island such as Great Mercury Island, Slipper Island and the Eastern Bay of Islands region (Matheson *et al.*2010, Turner and Schwarz *et al.*2006). Early European settlers and naturalists reported seagrass beds to be widespread in colonized coastal areas throughout New Zealand, further describing them as "very plentiful" towards the end of the nineteenth century (Inglis 2003, Morrison *et al.*2014). Extensive meadows were present in the Auckland area such as Waitemata Harbour, Tamaki Estuary, Okahu bay, Cheltenham Beach, and Manukau Harbour. Similarly, great expanses of seagrass beds were found in estuaries surrounding Whangarei, Tauranga, Porirua, eastern Bay of Islands, Golden Bay, Steward Island, Parengarenga and the Avon-Heathcote prior and during European settlement (Anderson *et al.*2019, Turner and Schwarz 2006).

In New Zealand, seagrass habitats act as biodiversity hotspots by supporting a range of taxonomical groups that rely on the numerous microhabitats that *Z. muelleri* provides. Communities typically consist of epiphytes, microfauna, sessile and mobile epifauna, infauna and epibenthic fauna (Table 1), however, the exact composition of the community will depend on local environmental conditions such as tidal force, sediment type and salinity and geographical positioning (Anderson *et al.*2019, Morrison *et al.*2014, Turner and Schwarz 2004). As seasons change, conditions acting upon estuarine and coastal environments fluctuate greatly. Physiographic characteristics in these habitat types result in a range of complex environmental gradients which promote high temporal heterogeneity (Thrush *et*

*al.*2013). The life cycles of species that reside along these environmental gradients will in turn reflect seasonal variation to optimise survival. Furthermore, studies have indicated that assemblage of both fish and benthic species are governed primarily by seasonal changes (Matheson *et al.*2014, Stål *et al.*2007). In New Zealand, seagrass production follows a clear seasonal pattern with above-ground biomass (grass shoots, excluding rhizomes and roots) highest during the summer months and lowest during winter months (Ramage and Schiel 1999, Turner and Schwarz 2006).

The profound role seagrasses have as a nursery for numerous fish species in New Zealand has only recently been acknowledged (Morrison *et al.* 2014, Turner and Schwarz 2006). Juveniles of commercially utilised species such as flounder, snapper, trevally, and many others have been associated with seagrass habitat in large numbers, in particular, subtidal seagrass where conditions are more benign and leaf blades are higher and denser (Morrison *et al.*2014). Experiments using artificial seagrass units confirmed that blade density and positioning within estuaries and harbours influence juvenile fish abundance (Parsons *et al.*2013). As these habitats influence the survival and growth of juveniles, we would expect the loss of nursey habitat to have consequences on adult fish populations, therefore, should be when determining the long-term stability of some commercially utilized species (Morrison *et al.*2014, Parsons *et al.*2013).

Table 1: Definitions and examples of community faunal groups associated with seagrass ecosystems.

Faunal groups	**Definition**	**References**
Epiphytes	Sessile, planktonic organisms that grow on seagrass leaves. Examples include algae (micro and macro), bacteria, fungi, bryozoans, and ascidians.	Brodersen *et al.* (2015), Larkum *et al.* (2006)
Microfauna	Eukaryotic, single celled organisms that are <100 μm in size. Examples include nematodes, arthropods, zooplankton.	Diadhiou *et al.* 2017, Walters and Moriarty (1993)
Sessile epifauna	Animals attached to seagrass leaves with no mobility throughout their life cycles. Examples include barnacles, mussels, and sea sponges.	Demers *et al.* (2016), Orth *et al.* (1984)
Mobile epifauna	Free moving animals that reside on leaves of seagrass beds. Examples include small crabs, sea stars, and shrimp.	Orth *et al.* (1984), Whippo *et al.* (2018)

Infauna	Organisms living with the sediment beneath and surrounding seagrass beds. Examples include gastropods, clams, and polychaetes.	Orth *et al.* (1984), Turner and Schwarz (2006)
Epibenthic fauna	Organisms that exist on or just above the sediment seafloor. Examples include fish and decapods	Orth (1984) Turner and Schwarz (2006)

While seagrasses are recognized as a temporary habitat for juvenile organisms, less attention has focused on the relationships between permanent fish inhabitants and their surroundings. Pipefish from the family Syngnathidae display a role-reversed mating system in which males take on the role of pregnancy (Braga Gonclaves *et al.*, 2016, Vincent *et al.*, 1995). Males develop specialized brooding structures that provide protection, aeration, and nutrients for developing embryos, whereas females may express colorful patterns, enlarged abdomens, or copulation dances to compete for males (Braga Goncalves *et al.*, 2016, Flanagan *et al.*, 2014 and 2016, Rose *et al.*2013, Vincent *et al.*, 1995, Wilson *et al.*, 2001). As permanent seagrass residents, pipefish are cryptic carnivores that use a specialized tubular snout to feed upon small crustaceans while remaining inconspicuous to predators by mimicking the leaf blades (Castro-Fernández *et al.* 2020, Garcia *et al.* 2005). Because of this, species of pipefish affiliated with seagrass habitats are predicted to be particularly susceptible to habitat fragmentation (Smith *et al.* 2010). In effect, pipefish distributions are predominantly influenced by a combination of structural complexity, predation pressure, and competition (Malavasi *et al.* 2007, Warfe and Barmuta 2004).

Objectives

Over the past decades, the global loss of seagrass has increased tenfold. Though well-established meadows are still prevalent in some regions, many species have experienced rapid die off from historic ranges as a result of environmental degradation and climate change (Orth *et al.*2006, Waycott *et al.*2009). The ecological importance of seagrasses to coastal communities and ecosystems has sparked a surge of comprehensive studies on the distribution, functionality, environmental limits and connectivity of seagrass habitat to adjacent ecosystems (e.g., coral reefs, salt marshes, mangroves etc) (Anderson et al 2017, Orth *et al.*2006, Turner *et al.*2006). Data compiled from 1128 observations of 215 seagrass sites throughout New Zealand all indicated some degree of decline with a cumulative loss of approximately 3370km2 (27km2 or 29% of loss per year) out of the 11592km^2 of seagrass meadows surveyed (Orth *et al.*2006). An overall total of 35% of loss recorded occurred from the 1980s onward, typically because of coastal development, dredging activities and declines in water quality (Waycott *et al.*2008). With only few comprehensive studies on current distributions and

limited reports of historical ranges, it is difficult to accurately determine changes over time. Because of this, studies have focused on mapping current distributions of seagrasses while less attention has been focused on the structure and dynamics of the associated fauna in association with local environmental conditions. (Anderson *et al.*2017, Matheson *et al.*2012 Reed *et al.*2004). This leaves a considerable knowledge gap in the ecological functioning of *Z. muelleri* ecosystems in New Zealand. Therefore, the main objectives of this study as demonstrated by chapters 1 and 3, were to assess spatial and temporal distributions of faunal species inhabiting seagrass beds across the South Island of New Zealand.

To date, very little has been done for comparing seagrass communities in the South Island (Morrison *et al.*2014, Turner and Schwarz 2006). In New Zealand, from the few studies available, most are conducted on meadows in the North island as they tend to be more widespread and closer to coastal development. This makes it easier to recognise change or when this habitat is struggling, whereas extensive seagrass beds of good condition in the South Island are typically located away from areas of large-scale urbanisation (Morrison *et al.*2014, Reed *et al.*2004). New Zealand for example, only has only one in depth report of the assemblage of juvenile fish species in living seagrass, however, this study was restricted only to subtidal seagrass beds from northern offshore islands (Morrison *et al.*2014). This means the use of seagrass beds by its community remain largely unknown in the South Island. To address this, chapter 1 covers faunal community assemblages from 9 seagrass beds across 3 regions (Nelson, Canterbury, Otago) within the South Island were evaluated with regard to latitude, local environment, and seagrass cover versus bare sediment. This allows for a clearer understanding of the degree to which faunal communities vary over space, with respect to local conditions.

Over time, marine organisms move between habitat types, be that daily, seasonally, or during different life cycle stages; therefore, seasonal changes in seagrass density coupled with fluctuations in environmental conditions are likely to drive biotic interactions (Jenkins *et al.*1998, Orth and Van Montfrans 1987, Turner and Schwarz 2006). We may expect seagrass communities to experience seasonal variations in behaviours such as habitat preference and reproductive timing, for example, juvenile fish densities in northern New Zealand were found to be highest during (February-April) (Matheson *et al.* 2014). During this time, subtidal beds may be more suitable providers of refugia as they are more environmentally benign and structurally developed compared to intertidal beds (Matheson *et al.* 2014). For example, subtidal habitat is characterised by having a higher density of longer leaf blades that reduces wave energy and increases the amount of surface area available for epifaunal grazing by up to 20% (Matheson *et al.*2014, Schwarz *et al.*2006). Currently, there is no available literature concerning temporal variations for seagrass communities in New Zealand. To address this, chapter 2 establishes seagrass associated faunal assemblages at Duvauchelle bay with respect to season, elevation, and seagrass cover versus bare sediment. By exploring dynamics over

time, we can gain a deeper understanding on how seagrass communities are influenced by seasonal changes in terms of their general abundance, life cycle stage and habitat preference.

Sygnathids have previously been used as keystone or flagship species for coastal habitat, particularly for seagrass as they are typically sensitive to changes in grass cover and bed composition (Shokri *et al.*2009). Through recent decades seagrass ecosystems that have experienced severe levels of human-induced degradation and decline, producing conditions may not be adequate to support pipefish populations (Scapin *et al.* 2018). Due to human interference, seagrass meadows have been subjected to a significant amount of habitat loss and fragmentation, resulting in an increased proportion of edge (Boström *et al.*, 2006, Smith *et al.*, 2008, Tanner, 2005). While increased edges may suggest more available habitat for those that utilise these areas, there is also the issue of these individuals being more vulnerable to edge related disturbance (Smith *et al.*, 2008, Tanner, 2005). As *S. nigra* have been observed utilising seagrass edge habitat, it is likely they will be negatively impacted by edge effects if seagrass decline continues (Kendrick and Hyndes 2005, Smith *et al.* 2008, Tanner 2005). For instance, as a permanent seagrass inhabitant, *S.nigra* heavily relies on seagrass leaves for attachment and camouflage, in addition to provision of resources, breeding and nursey grounds. Consequently, if a seagrass habitat is rapidly displaced by means of extreme climatic events or human interference, residential pipefish species will experience severe negative implications for survival (Arias-Ortiz *et al.* 2018, Scapin *et al.* 2018).

Apart from two published studies literatures concerning native pipefish in New Zealand is limited to presence/absence data and tend to focus on broader subjects such as overall fish community assemblage and abundances (Dawson 1982, Parsons *et al.*, 2014, Schwarz *et al.*, 2006). Timing of reproduction and habitat choice may resemble Australian populations, however ocean waters in New Zealand are on average much colder and may facilitate variation in life history traits. Because of this pipefish may only breed during warmer months as opposed to all year round (Dawson 1982, Roberts *et al.*2015). With seagrass habitats in a global regression, it is pertinent to determine the interactions between distributions of threatened species and local habitat traits (Castro-Fernández *et al.* 2020). Therefore, the last objectives of this study were to examine population dynamics over time, space, and seagrass cover, in conjunction with determining the breeding ecology of New Zealand's native pipefish species at Duvauchelle bay. In doing this, we aim to gain insight into the general ecology and distribution of an understudied yet fascinating taxa.

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Chapter 2

Assessing temporal changes in community composition and assemblage of seagrass beds at Duvauchelle bay

2.1 Introduction

Seagrass systems are recognised worldwide as a major enhancer of coastal biodiversity. Nonetheless, investigation into the effect of seagrass over time and space on faunal compositions often demonstrate highly variable results (Turner et al 1999, Xu *et al.* 2016). Seagrasses experience localized changes in biotic conditions as dictated by the ebb and flow of the tide, in conjunction with freshwater ways that deliver terrestrial inputs (Orth *et al.* 1984). The mixing of fresh and saltwater, in conjunction with daily fluxes in desiccation stresses such as temperature and light present profound physiological challenges for associated biota (Tagatz and Marlin 1971, York *et al.* 2018). Thriving in brackish waters requires organisms to have specialised mechanisms to tolerate rapid changes in salinity and temperature (Tagatz and Marlin 1971, York *et al.* 2018). For example, bivalves such as mussels, oysters, and clams display behavioural responses by closing their shells and switching to aerobic respiration during low tide. Other sessile organisms, seagrasses, for example, possess unique morphological and physiological adaptations to withstand various degrees of salinity and temperature (Demers *et al.* 2016, York *et al.* 2018).

In New Zealand, seagrass systems are characterized by the single species *Zostera muelleri* which is predominantly distributed along intertidal zones, forming extensive patches throughout the mid to low tidal lines (Morrison *et al.* 2014, Turner and Schwarz 2006). Beds may also extend into permanently submerged subtidal fringes though are primarily constrained by light availability (Larkum *et al.* 2006). Thus, seagrass beds within a single bay are subject to a varying range of biotic conditions brought on by daily tidal fluxes (Larkum *et al.* 2006, Whippo *et al.* 2018). Furthermore, exposure to consistent changes in conditions suggests that seagrass communities require assessment of both spatial and temporal aspects for a comprehensive representation of associated faunal assemblage (Whippo *et al.* 2018). In temperate regions, seasonal changes in temperature and seagrass cover have been attributed to influencing abundance and species replacement (Wu *et al.* 2018). For example, the functioning of seagrass species, including *Z. muelleri,* as nursery habitat incorporates strong seasonal patterns in recruitment of fish species where abundance and richness peaks in summer, through to early autumn (Mateo and Tobias 2008, Morrison *et al.* 2014, Wu *et al.* 2018). Faunal compositions both within and between seagrass systems are inherently subject to seasonal changes in abundance and species replacement, thus incorporating temporal aspects is vital to fully characterize seagrass systems.

Seagrass habitat throughout New Zealand has followed global trends of rapid decline in response to anthropogenic disturbance (Gastron 2000, Turner and Schwarz 2006). Numerous studies have reported that meadows of higher structural complexity, that being of higher shoot density, canopy height, and left area provide invaluable habitat for a broad range of fish and invertebrate communities compared to those of low complexity (Bostrom *et al.* 2006, Cullen-Unsworth *et al.* 2014, McClosket and Unsworth 2015). *Z. muelleri* ecosystems in New Zealand play pivotal roles in estuarine ecological functioning and in supporting a myriad of associated organisms, yet to date, no studies have explored fine-scale Spatio-temporal variation in infaunal communities (Morrison *et al.* 2014). Moreover, the lack of long-term monitoring induces considerable uncertainties on the natural dynamics of *Z. muelleri* communities Smale *et al.* 2019). To infer how anthropogenic pressures influence biodiversity it is essential to establish a baseline understanding of the natural dynamics between seagrass beds and their associated communities (York *et al.* 2018).

While small-scale habitat variations are common place, human induced disturbance can intensify these changes to a point that may diminish the ability of habitats to recover (Guidetti and Bussotti, 2000, McClosket and Unsworth, 2015). Given their shallow water distributions, seagrass meadows are an example of an ecosystems where natural and anthropogenic forces can lead to fragmentation (McClosket and Unsworth, 2015). Species assemblage by far, is considered the best descriptor and response variable to estimate the impact of environmental changes in ecosystems (Boström *et al.* 2006, Gastron 2000, Whippo *et al.* 2018). Factors such as temperature, salinity, sedimentation,

seagrass cover or disturbance are frequently used to explain spatial patterns for seagrass associated faunal communities (Al-Wedaei *et al.* 2018, Whippo *et al.* 2018, Xu *et al.* 2016).

Behavioural aspects such as migration for predator avoidance, reproduction and food availability also play a role in establishing diversity patterns through time and space (Crist and Veech 2006, Henriques-Silvia *et al.* 2013). For example, fish species in New Zealand such as blue cod, snapper, trevally, and leather jackets were found utilising *Z. muelleri* habitat for resource and refuge during juvenile life stages (Morrison *et al.* 2014, Tunrner and Schwarz *et al.* 2006).

The aim of this study is to establish a baseline understanding of how faunal community composition is influenced by space, time and seagrass cover. First, we hypothesize that faunal communities will follow a depth gradient, taxonomic diversity and composition will be highest throughout subtidal zones and lowest throughout intertidal zones. Secondly, we hypothesize that faunal communities will display seasonal variation that will increase during the warmer spring and summer months and drop during winter and fall months. Lastly, seagrass patches will support a more abundant faunal community compared to neighbouring unvegetated habitat, with dense patches supporting the highest abundances.

2.2 Methods and materials

2.2.1 Study site

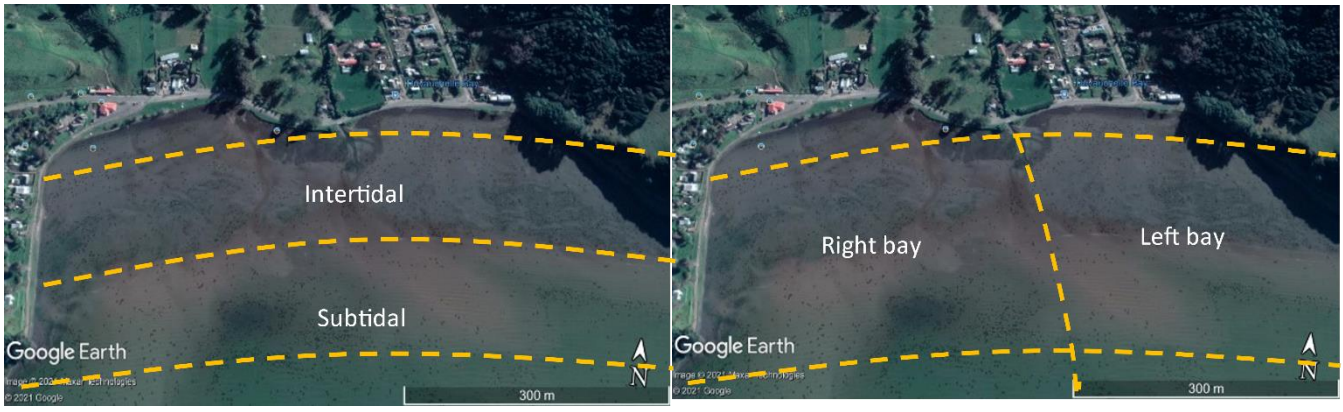


Figure 1: Aerial image of Duvauchelle bay. For analyses the bay was sectioned into zones that represented tidal elevation (a. subtidal, intertidal) and bay position (b. right, left) to examine localized changes in community composition over the seasons.

This study was conducted in Duvauchelle bay (43.7504° S, 172.9334° E), located in the Akaroa Harbour of New Zealand. Prior to sampling, the location of the seagrass beds was assessed at low tide to establish appropriate sampling sites. Duvauchelle bay is approximately 800km across and extends a

further 1k m into the Akaroa harbor. Vast seagrass beds exist throughout the bay in a mosaic that ranges from sparse to highly dense seagrass cover (fig 2) and provides an ideal site for assessing temporal changes in seagrass communities across different elevations and sites. To assess temporal variation in community structure, monthly from November 2019 to October 2020. The methodology used for each sampling trip consists of: (1) sampling seagrass beds and bare sand patches using a seine net;(2) identifying and counting all organisms caught and (3) measuring species of interest to determine adult and juvenile life stages.

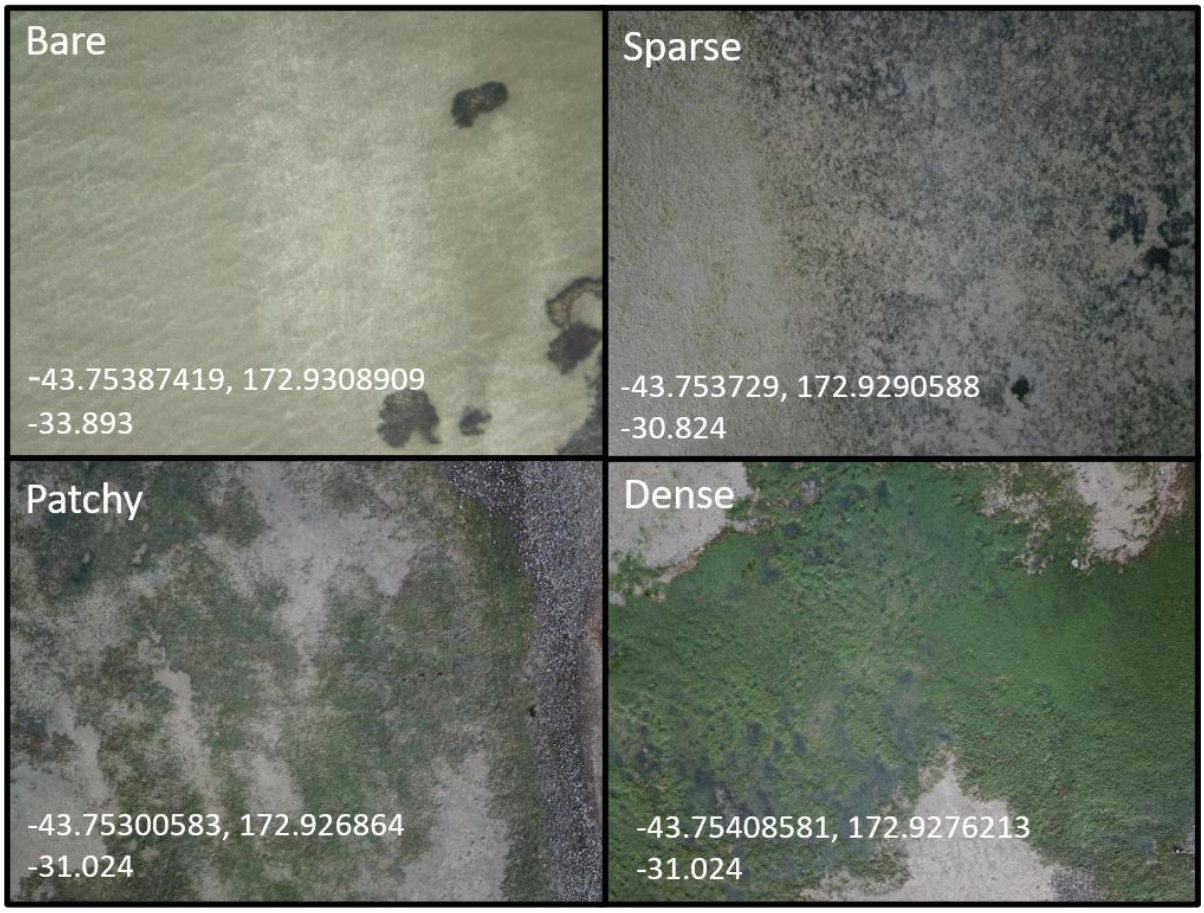


Figure 2: Aerial drone shot representations of seagrass cover densities for sampling. Also included are geographical coordinated in latitudinal/longitudinal form and the geometric altitude (z).

2.2.2 Field sampling

Data loggers (HOBO and InTemp data loggers, Onset) were deployed before sampling for recording changes in light and temperature at high, mid, and low tidal zones on both sides of the bay (fig 1). Duvauchelle bay was sampled once per month for a year and consisted of 12 daily sampling events in total, excluding the month of March 2020. Sampling typically commenced in the mid-to-late morning in the upper/mid tidal zones several hours before low tide and continued into the subtidal zones as the tide receded. In doing this, we have a time efficient method to sample both intertidal and subtidal zones while also avoiding any debris or unrelated species from the incoming tide. This method does

however have time constraints as Duvauchelle bay drains rapidly after reaching roughly half tide. Because of this, some sampling events did not include replicates from both sides of the intertidal zone.

A modified 1.2m x 1.2m seine net mounted on a PVC frame was hand-hauled, parallel to the shore in a series of non-overlapping pulls throughout the high, mid, and low tidal zone on both sides of the bay. At the start and end of each tow, coordinates and time were recorded using a Garmin GPS and seagrass density was visually scored as sparse, patchy, dense, or bare substrate (fig. 2). Tows typically ranged between 5-20m, though this is not the case for all samples. This method had minimal to no destructive impact in terms of uprooting seagrass plants (Thomsen *et al.* 2020). For each sampling trip, the goal was to cover certain positions of the bay so that each season had at least 60 observations, with 30 tows in each general position of the bay (fig.1).

At the end of each tow, organisms were identified at the lowest taxonomic level, counted, recorded, and some species, including fish, and taxa that displayed large size ranged were measured to give an indication of life stage (eg. Juvenile, adult). Any unidentifiable organisms or those of interest were photographed for future processing. For logistical ease, shrimp individuals caught were identified and expressed as shrimp spp.

2.2.3 Data analysis

Prior to analysis, raw counts were standardized into a measure of species density or units per area to deal with differences in sampling effort. Sample area was calculated by taking the length of each tow (using Garmin GPS coordinates) and multiplying by the width of the net (1.2m). Monthly data was further pooled into seasonal abundance (Summer – December 2019, January 2020, February 2020, Autumn – 1st May 2020, 26th May 2020, Winter – June 2020, July 2020, August 2020, Spring – October 2020, September 2020, November 2020). Summary statistics were generated to examine changes in individual density over tidal elevation and season. Secondly, tows were generated into heatmaps, using the top 15 most abundant species to examine seasonal changes in dominance. To further explore aspects of dominance, species were pooled into taxonomic units as converted into proportions.

Field sampling is labour intensive and the majority of biodiversity studied almost always miss unseen species, especially seeing as faunal communities are often defined by a few highly abundant dominant species, alongside numerous rare species (Chao *et al.,* 2009). Estimating species richness where rare species represent a substantial portion of the community poses a statistical challenge as is it seldom possible to obtain enough samples to fully characterize all species that are present (Chao *et al.* 2009).

To compare sample coverage and diversity across tidal elevation and seasons, single tows were pooled into seasonal observations for spring, summer, autumn and winter, and standardized by completeness rather than size using the package *iNEXT*, as recommended by Chao and Jost (2012).

Repeated-measures ANOVA were performed to test for discrepancies in community composition, relative to bay position (left or right), and elevation (subtidal or intertidal), which were included as categorical covariates. For each season, alpha and beta diversity following Marcons (2012) decomposition of Shannon's entropy were calculated to detect compositional changes between sites and seasons. Discrepancies of alpha diversity calculated using Macron and Herault's (2012) asymptotic estimators of unseen species were further tested across season, elevation, and bay side with repeated measures ANOVA, including season: elevation and season: bay position interactions.

Approximate estimates of species richness were generated using the rarefaction measure, proposed by Heck *et al.* (1975) and is defined as follows:

$$E(S) = \sum_{i=1}^{S} \left(1 - \left[\frac{\binom{N - N_i}{n}}{\binom{N}{n}} \right] \right)$$

Where *S* is the number of species, *n* is the standard sample size used for comparison, and N and N_i corresponds to the total sample size, and number of individuals in the i^{th} species (Chao 2012). Observed and extrapolated richness (q=0) and other factors of q (1,2) were plotted across sites and seasons. To standardize for sampling effort between seasons when calculating diversity, asymptotic estimators were used to measure unseen species, based on the proportion of rare species in the original data and species accumulation plots based on extrapolated diversity values were generated (Chao et al 2009, Macron and Herault 2021).

Aspects of diversity were explored using a range of methods that partition diversity into ecological relevant components. Estimates of taxonomic diversity were based on Hill numbers, otherwise known as the effective number of species. As developed by Hill (1973), Hill numbers quantify diversity into equivalent numbers of equally abundant species and allows for species abundance to be weighted exponentially by the factor *q* and is defined as follows:

$$D^q = \left(\sum_{i=1}^{S} p_i^q \right)^{1/(1-q)}$$

Where *S* is the species number, p_i corresponds to the abundance of the *i*th species, and *q* represents sensitivity to species abundance (Merderos *et al.* 2021). As *q* increases, effective weight was assigned

to more abundant species, thus, indicating the diversity of dominant species as rare species become suppressed (Chao *et al.* 2014). This parameter specifies the sensitivity of relative abundances, where q=0, q=1, and q=2 represents species richness, Shannon's entropy, and Simpson's diversity respectively (Chao and Jost 2010). Additionally, unlike traditional diversity indexes, Hills numbers satisfies the principle of replication (Chao *et al.* 2014, Hill 1973). Using the function DivEst () from the package enteropart, components of diversity (gamma, alpha, beta) across factors of q (0,1,2) were partitioned over 100 simulations and while using the ChaoJost correction for unseen species (Macron and Herault, 2021). Alpha diversity at q=0 was then plotted with confidence interval to assess how mean diversity changes across seasons and tidal elevations.

Two approaches (multiplicative and additive) were applied to examine patterns in beta diversity. The multiplicative approach follows Jost *et al.* (2010) decomposition of diversity, where beta = gamma/alpha which can be interpreted as regional species diversity or the effective number of unique faunal communities present within a system. For this metric, alpha and beta were treated as independent components, while considering rare (${}^{0}D$), typical (${}^{1}D$), and dominant (${}^{2}D$) species to describe compositional differentiation among communities (Chao *et al.* 2014, Jost 2007, Jost *et al.* 2010). The degree and magnitude to which tidal elevation and bay side contribute to beta diversity were further analyses using principle components analysis and plotted in multidimensional space.

For the additive approach, pairwise beta-diversity values were used to generate components of nestedness and species turnover between sites and seasons (Baselga 2010, Medeiros *et al.* 2021). Beta diversity is of great interest to ecologist as it reflects the processes that generate and maintain variation in communities within a single region (Whittaker 1960).

Recent advances in the computation of beta diversity have allowed turnover to be partitioned into measures of nestedness, species replacement, and differences in richness (Baselga 2010). Nestedness incorporates the degree to which the composition of smaller samples will be encompassed by larger samples that have higher richness (Baselga 2010, Ulrich and Gotelli, 2007). Secondly, species turnover measures which samples do not share species, and how they differ among samples. To obtain such components, among-sample Sørensen's dissimilarity was measured, then the Simpson's dissimilarity index (species turnover) was subtracted to give a measure of nestedness (Baselga 2010, Ulrich and Gotelli, 2007, Whittaker 1960). Partitioning of beta diversity into nestedness and replacement components was achieved following Baselga (2010) method of generating measures of nestedness and turnover, based on Sørensen and Simpsons multiple-site dissimilarity:

$$\beta_{SOR} = \frac{\left[\sum_{i<j} \min(b_{ij}, b_{ji})\right] + \left[\sum_{i<j} max(b_{ij}, b_{ji})\right]}{\left[\sum_i S_i - S_T\right] + \left[\sum_{i<j} \min(b_{ij}, b_{ji})\right] + \left[\sum_{i<j} max(b_{ij}, b_{ji})\right]}$$

$$\beta_{SIM} = \frac{\left[\sum_{i<j} \min(b_{ij}, b_{ji})\right]}{\left[\sum_i S_i - S_T\right] + \left[\sum_{i<j} \min(b_{ij}, b_{ji})\right]}$$

Where S_i is the total number of species in a particular site (*i)*, S_T is the total number of species over all sites, and b_{ij}, b_{ji} represents species that are exclusive to sites *i* and *j* sequentially. Estimates of alpha, gamma, beta and further components were generated in R using the ChaoJost asymptotic estimator for unseen species from the package *entropart* (Macron and Herault, 2021). All analysis was performed using the statistical program R (Rstudio Team 2020).

2.3 Results

Over 12 sampling events and 401 tows, 31 species were identified with a total of 24,097 individuals caught. Of those 31, shrimp spp, sand flounder, and black top-shell were consistently the most abundant species across sites and seasons which contributed to 67.5%, 10.72%, and 0.06% to overall abundance respectively. All other species caught had a total abundance under 1000 individuals, thus, we can infer that community abundance in Duvauchelle bay is dominated by the species listed above. While most species were present across elevations, overall abundances and density were markedly higher in the subtidal zone, with the exception of sand flounder (fig. 3, fig.4). Across seasons, individual density peaked during the months of autumn and spring whereas, both subtidal and intertidal elevations display significant reductions in faunal community abundance during the summer. When comparing between elevation and seasons, outliers for the intertidal zone during the

summer were not as varied (fig. 3)

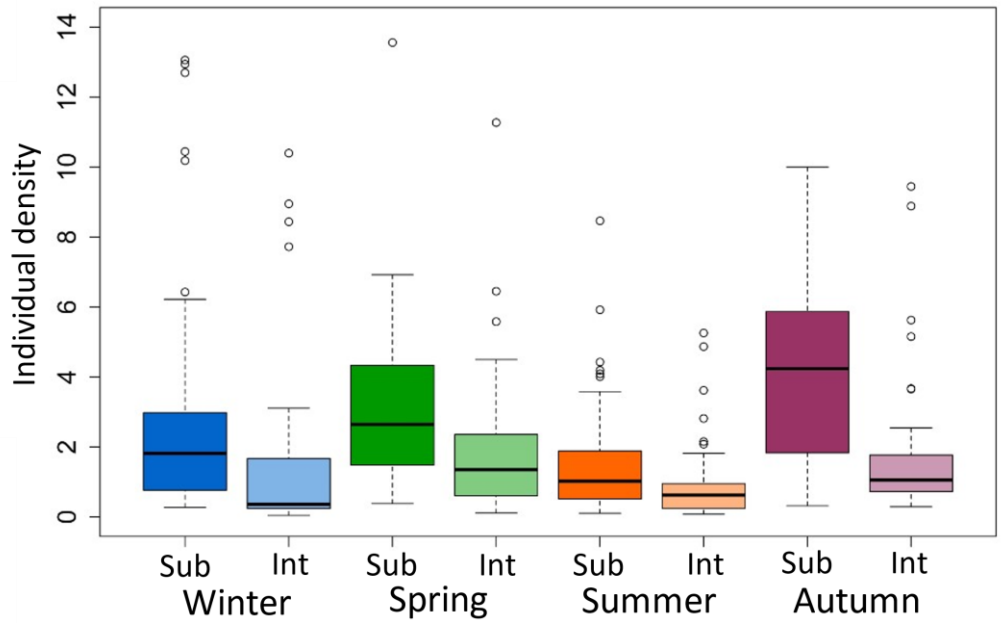
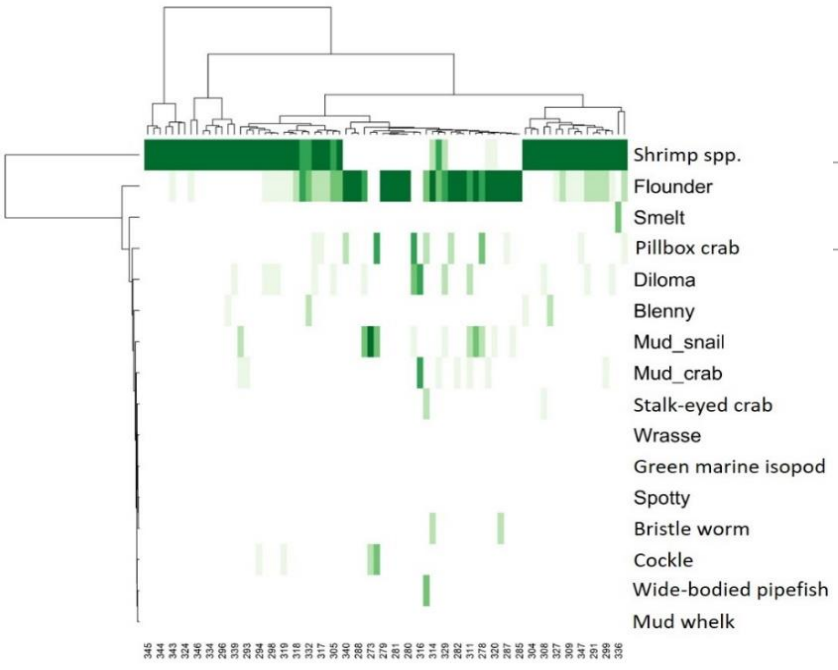


Figure 3: Boxplots showing summary statistics of individual density across seasons and elevations, where sub represents subtidal and int represents intertidal. Abundance values were divided by the area (m2) of respective tows and expressed as a measure of species

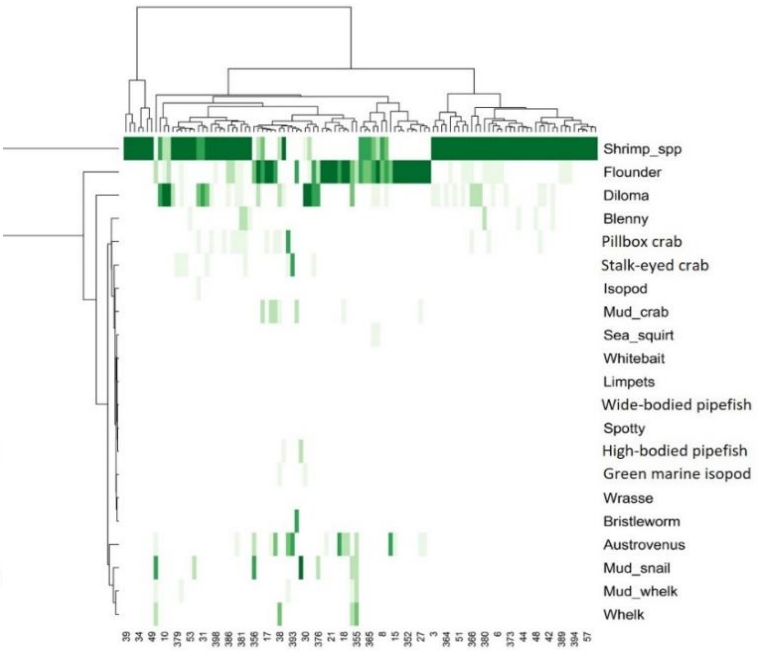
Table 2: Total and average [] density of species per tidal elevation and season. Raw counts were converted into a measure of individual density where abundances were divided by sampling/tow area (m2). Densities were further divided by the number of samples to calculate average density. Sections correlate to groupings of taxonomic classes (bony fish, crustaceans, gastropods, bivalves, misc. invertebrates).

	Winter		Spring		Summer		Autumn	
	Int	Sub	Int	Sub	Int	Sub	Int	Sub
Sand flounder (+) (*)	49.72 [1.212]	14.45 [0.35]	29.64 [0.471]	13.41 [0.02]	2.49 [0.037]	1.58 [13.41]	9.78 [0.19]	13.34 [0.27]
Blenniiformes (+) (*)	0.27 [0.006]	1.77 [0.04]	2.28 [0.036]	4.52 [0.51]	0.69 [0.011]	6.75 [0.10]	3.47 [0.069]	7.83 [0.16]
Blue cod (*)	-	0.03 [0.0006]	-	-	-	-	-	0.211 [0.004]
White bait	-	-	0.15 [0.002]	0.18 [0.017]		0.23 [0.003]	-	0.06 [0.001]
Kahawai	-	-	0.05 [0.0007]	-	0.82 [0.012]	1.60 [0.02]	-	-
Smelt	0.26 [0.006]	2.88 [0.07]	-	-	-	-	-	0.08 [0.002]
Banded wrasse (*)	0.22 [0.005]	0.68 [0.016]	0.21 [0.003]	0.74 [0.007]	-	0.73 [0.011]	0.12 [0.002]	1.98 [0.039]
Spotty (+) (*)	-	-	0.09 [0.0001]	-	-	-	0.21 [0.0154]	-
Wide-bodied pipefish (*)	0.24 [0.0058]	0.76 [0.02]	0.03 [0.004]	0.72 [0.71]	0.15 [0.0022]	0.41 [0.01]	0.28 [0.005]	0.38 [0.01]

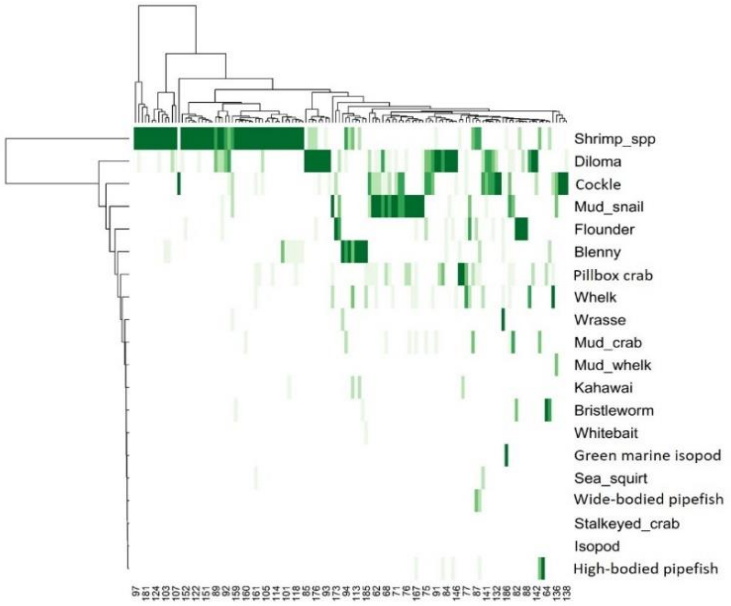
High-body pipefish	-	-	0.04 [0.0005]	1.98 [1.98]	0.32 [0.01]	0.35 [0.005]	-	0.11 [0.002]
Shrimp *spp.* (+)	44.70 [1.09]	107.09 [2.61]	35.96 [0.57]	139.85 [2.22]	9.46 [0.143]	72.81 [1.10]	39.73 [0.795]	160.78 [3.22]
Pillbox crab (+)	11.89 [0.29]	5.03 [0.122]	4.10 [0.065]	5.01 [0.079]	5.89 [0.089]	2.37 [0.035]	3.65 [0.073]	8.06 [0.16]
Burrowing mud crab	0.78 [0.019]	1.26 [0.03]	1.56 [0.024]	1.56 [0.024]	1.22 [0.0185]	0.7 [0.01]	0.95 [0.0189]	1.67 [0.034]
Stalk-eyed mud crab (+)	0.14 [0.003]	0.74 [0.017]	2.12 [0.0337]	2.03 [0.024]	0.28 [0.004]	0.03 [0.011]	0.40 [0.008]	0.83 [0.034]
Swimmer crab	-	0.024 [0.00005]	0.02 [0.0003]	-	-	-	-	-
Pie crust crab	-	0.10 [0.0025]	0.04 [0.0006]	-	-	-	-	-
Hermit crab	-	-	-	0.081 [0.001]	-	-	-	0.02 [0.00004]
Isopod *spp.*	-	-	1.50 [0.023]	1.12 [0.017]	-	0.12 [0.0017]	-	-
Sea centipede (+)	0.26 [0.006]	0.77 [0.018]	0.65 [0.009]	1.57 [0.025]	-	0.19 [0.0028]	0.30 [0.006]	1.71 [0.034]
Mudflat top shell (+)	2.18 [0.053]	2.63 [0.064]	9.80 [0.156]	13.65 [0.22]	12.25 [0.185]	6.48 [0.098]	1.63 [0.0327]	2.79 [0.056]
Mud snail	0.95 [0.023]	0.43 [0.01]	1.73 [0.027]	4.81 [0.076]	8.81 [0.1334]	1.26 [0.019]	0.38 [0.0076]	0.44 [0.008]
Cats eye	-	-	-	-	-	-	-	0.03
Limpet	-	-	0.02 [0.0002]	0.17 [0.003]	0.03 [0.0004]	-		-
Mud whelk	0.44 [0.0107]	0.52 [0.013]	1.26 [0.02]	1.34 [0.765]	0.20 [0.003]	-	0.11 [0.002]	0.45 [0.0088]
Southern creeper (+)	-	-	1.06 [0.0169]	1.56 [0.025]	2.87 [0.0435]	0.71 [0.0108]	0.32 [0.0064]	0.79 [0.0157]
Cockle (+)	0.88 [0.214]	1.15 [0.027]	3.52 [0.056]	2.24 [0.035]	8.78 [0.133]	0.64 [0.0096]	1.22 [0.0245]	2.54 [0.051]
Venus clam	-	-	0.02 [0.0003]	-	-	-	-	-
Pipi	-	-	-	-	0.02 [0.0002]	0.02 [0.00029]	-	0.04 [0.0007]
Cushion star	-	-	-	-	-	-	-	0.03 [0.00005]
Sea squirt	-	-	0.39 [0.0062]	0.24 [0.0038]	0.14 [0.002]	0.16 [0.0024]	-	-
Bristleworm	0.21 [0.005]	0.45 [0.012]	0.24 [0.0038]	0.88 [0.014]	0.3 [0.0045]	0.51 [0.0077]	0.14 [0.0027]	0.14 [0.0027]



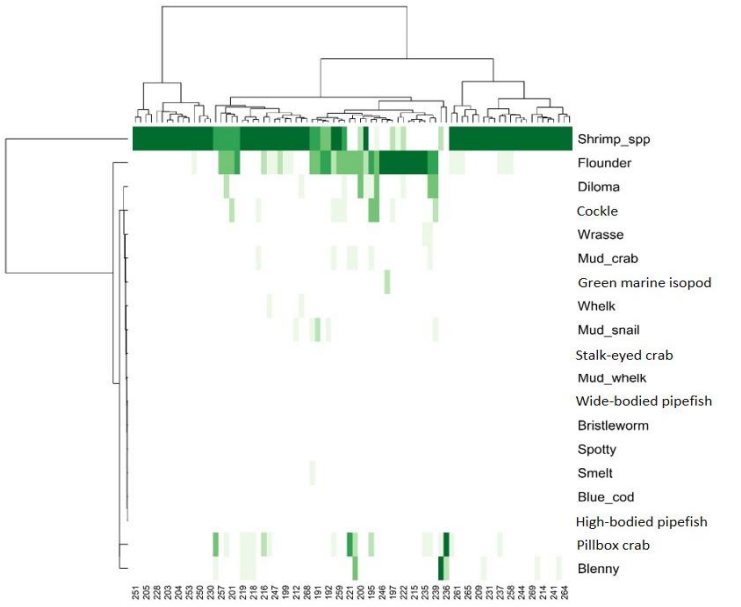
Winter



Spring



Summer



Autumn

Figure 4: Community composition heatmaps across seasons, calculated using the Bray-Curtis dissimilarity matrix. Shades of green represent species abundance relative to observations, where darker shades represent high abundances and vice versa. Species are ordered by overall abundance as indicated by the dendrogram on the y axis, thus, higher abundance taxa are positioned at the top of the graph. Observations are clustered by compositional similarity, hence, observations with the same or similar species group together, as illutrated by the top dendrogram.

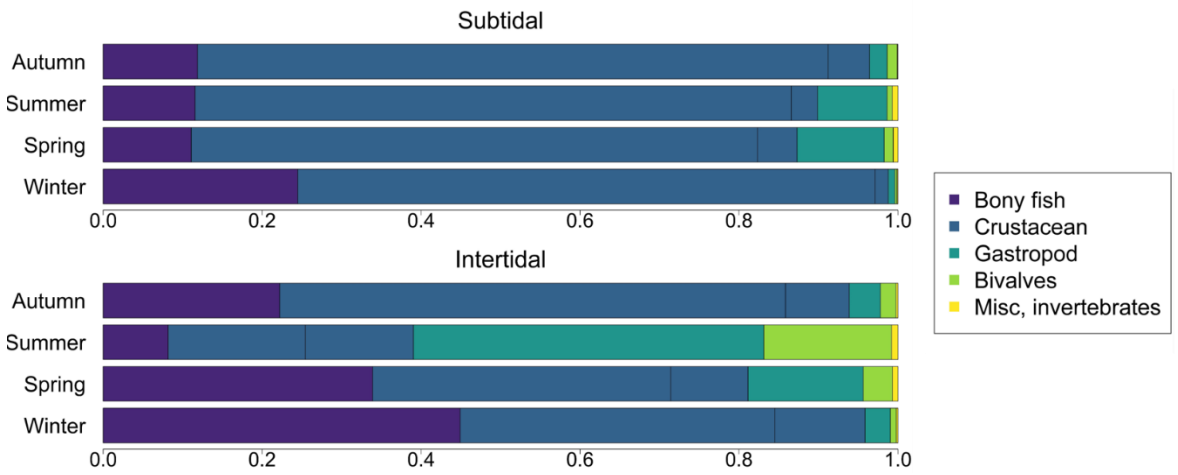


Figure 5: Proportional density of taxonomic classes across seasons and elevation. Proportional density of shrimp has been included and is represented by the first section in the crustacean class. Three species of different taxonomic class that occurred in very small numbers, thus, for plotting purposes, counts were pooled together and classed as miscellaneous invertebrates. Species included bristleworms, seastars, and sea squirts. Abundance counts for each class were then standarized by sample area and converted into proportions.

Overall, proportional density of taxonomic classes was dominated by and bony fish (fig. 5). Shrimp remain the dominant species across seasons with the exception of the intertidal zone during summer (table 2, fig 5). Here, the community is dominated mostly by gastropod taxa which represents 45.19% of the community sampled, through the majority of individuals present were either black top-shell (498 individuals caught) or mud snail (233 individuals caught). During this time bivalves also contribute more during this time, though, this is likely due to the heavy reduction in the abundance of highly common species such as shrimp and sand flounder. Throughout the year, bony fish in the subtidal zone occur at similar intervals from autumn to spring and spiking in winter. Throughout the winter months, dominant fish species in ranking order include sand flounder (592 caught), blenny (70 caught), smelt (62 caught) wrasse (28 caught), wide-bodied pipefish (22 caught), spotty (11 caught) and blue cod (1 caught).

Table 3 Total and average [] density of taxonomic classes per tidal elevation and season. (See table 2 for species groupings). Raw counts were converted into a measure of individual density where abundances were divided by sampling/tow area (m2). Densities were further divided by the

	Winter		Spring		Summer		Autumn	
	Int	Sub	Int	Sub	Int	Sub	Int	Sub
Bony fish	50.71 [1.236]	20.87 [0.509]	32.5 [0.515]	21.74 [0.345]	4.78 [0.067]	11.22 [0.17]	13.87 [0.277]	24.07 [0.48]
Crustacean	57.78 [1.409]	115.01 [2.805]	45.93 [0.729]	151.22 [2.4]	16.91 [0.256]	76.21 [1.1548]	45.04 [0.9007]	173.09 [3.46]

Gastropod	3.58 [0.087]	3.59 [0.087]	13.89 [0.22]	21.55 [0.342]	24.17 [0.366[	8.47 [0.128]	2.46 [0.049]	4.5 [0.09]
Bivalves	0.88 [0.021]	1.45 [0.0279]	3.547 [0.056]	2.24 [0.035]	8.8 [0.133]	0.68 [0.0099]	1.22 [0.024]	2.57 [0.051]
Misc. invertebrates	0.21 [0.005]	0.45 [0.0109]	0.64 [0.01]	1.12 [0.017]	0.44 [0.0066]	0.67 [0.01]	0.14 [0.002]	0.16 [0.003]

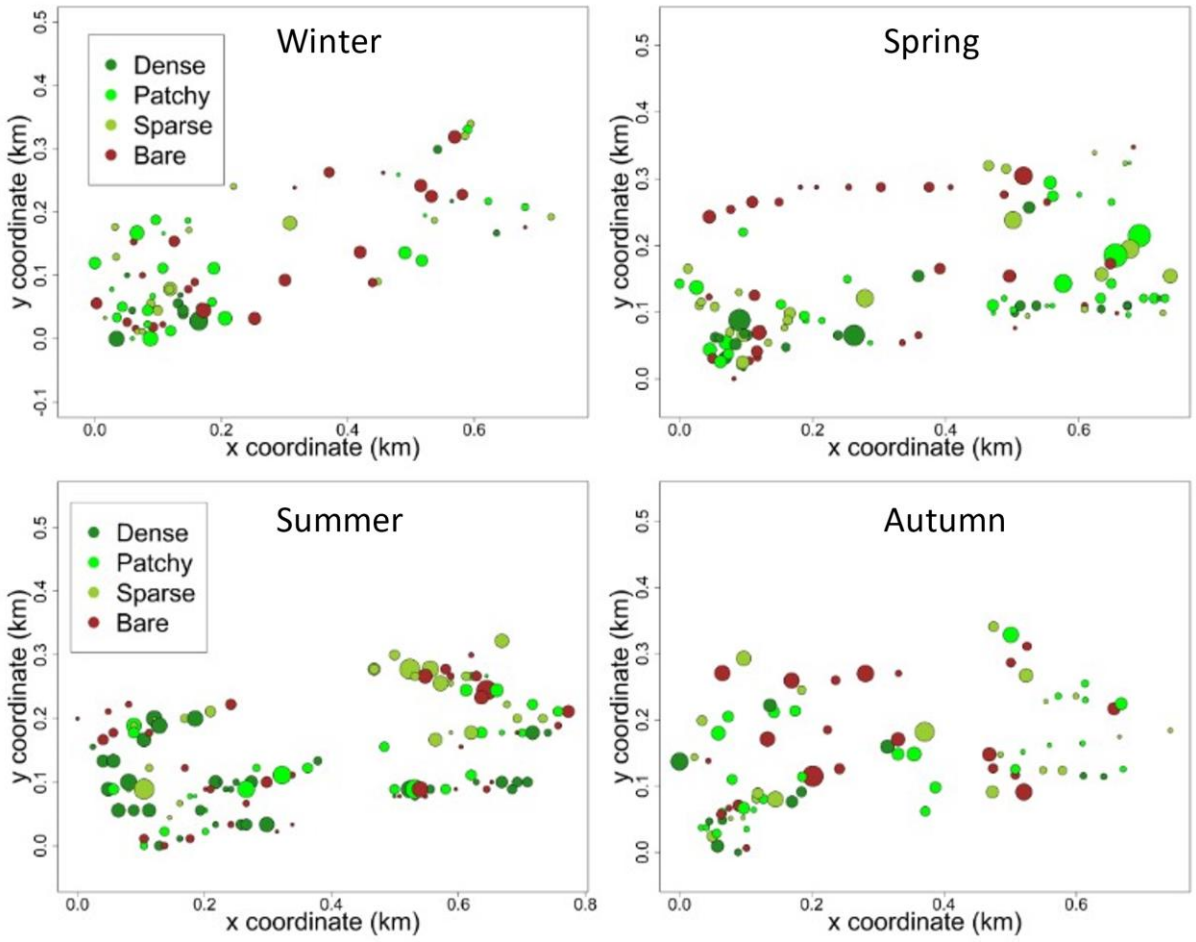


Figure 6: Alpha diversity plotted with geographic position of corresponding tows across seasons. Elevation in each plot is defined by a dotted line, where below the line represents the subtidal zone and above the line represents intertidal. Point size is proportional to alpha, thus, larger points correspond to sites that have higher average diversity. Colours represent categorical representations of seagrass density for each observation.

While there are no significant differences for alpha diversity between between seasons, figure 6 suggests that alpha diversity was, on average higher in patchy-dense seagrass patchches throughout the subtidal zone. Across replicates of bare sediment (brown) average effective species appears less so than that of patchy and dense replicates.

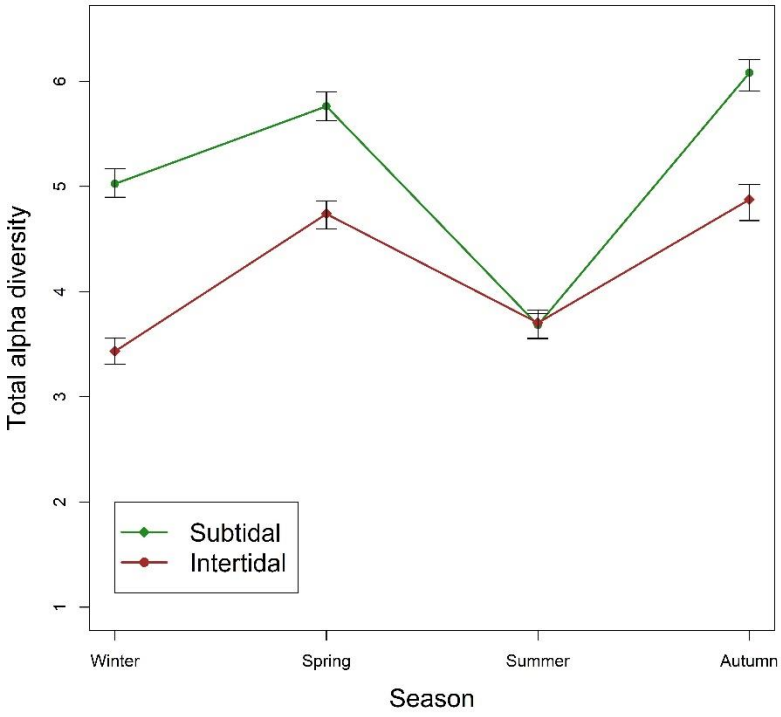


Figure 7: Seasonal variations of partitioned alpha diversity for subtidal and intertidal zones of Duvauchelle bay. Calculations for alpha diversity follow Marcons (2012) decomposition of Shannon's entropy into diversity components. Data was first converted into a metacommunity object which was partitioned into components of diversity (alpha, beta, gamma) and estimated confidence intervals over 1000 simulations.

Estimates of alpha diversity vary significantly between subtidal and intertidal elevations. Alpha diversity across subtidal zone increased markedly into the spring and autumn months, peaking with a mean richness of 6.143 per sample for pooled abundances. Overall, subtidal samples contained the most diverse and abundant communities, however, abundances were heavily driven by three highly abundant species, those being shrimp (*Palaemon* spp.), juvenile sand flounder (*Rhombosolea plebeia*), and top-shell (*Diloma* spp.) (Fig. 2).

As shown by figure 7, total alpha diversity peaked during the autumn for both intertidal and subtidal zones, followed by spring then winter which both followed a similar elevational pattern, whereas diversity for the subtidal zone in the summer displayed a particularly notable drop. One particularly surprising finding as shown by figure 7 was the highly similar estimation value for alpha diversity between elevations during the summer, followed by a marked increase during the fall. When examining alpha across single sampling events rather than season, we see that the overall reduction seen in the summer months can be attributed to the month of January. For example, from December to January, the overall catch went from 1806 individuals to 640, yet both months share the same species richness of 17. Species composition varies only slightly between the two months with species such as *Leptonotus elevatus*, whitebait spp., and isopod spp. Overall, season (p=0.034) had more influence on

alpha diversity than tidal elevation (p=0.218) or bay position (p=0.0794). When analysed as independent components, bay side explains more variation the elevation, however, there is a notable interaction between elevation and season which suggests that alpha diversity across elevations are subject to change over time.

Table 4: Repeated measures analyses of alpha diversity across seasons, elevation, and bay position. Alpha diversity for each observation was computed following Marcon's (2012) decomposition of Shannon's entropy into diversity components.

	F-value	P-value
Elevation	1.5163	0.2189
Bay position	3.0931	0.0794
Season	2.9328	0.0334
Elevation: Season	3.7316	0.0115
Bay position: Season	1.7794	0.1505

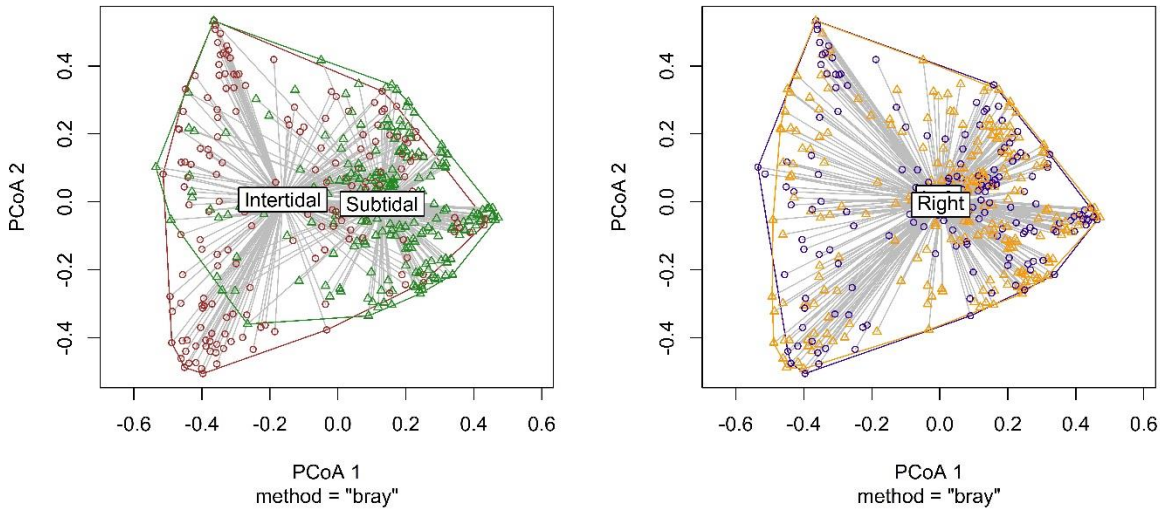


Figure 8: Ordination variations in beta diversity for elevation and bay position. Between tidal zones there is a considerable degree of separation between intertidal and subtidal communities whereas bay sides show no significant groupings. This suggests that spa

As shown in figure 8, clusters for the right and left side of the bay suggest that both positions share significant similarities in shared species. Subtidal and intertidal zones show more differentiation that bay position, however, each elevation still shares a considerable amount of species.

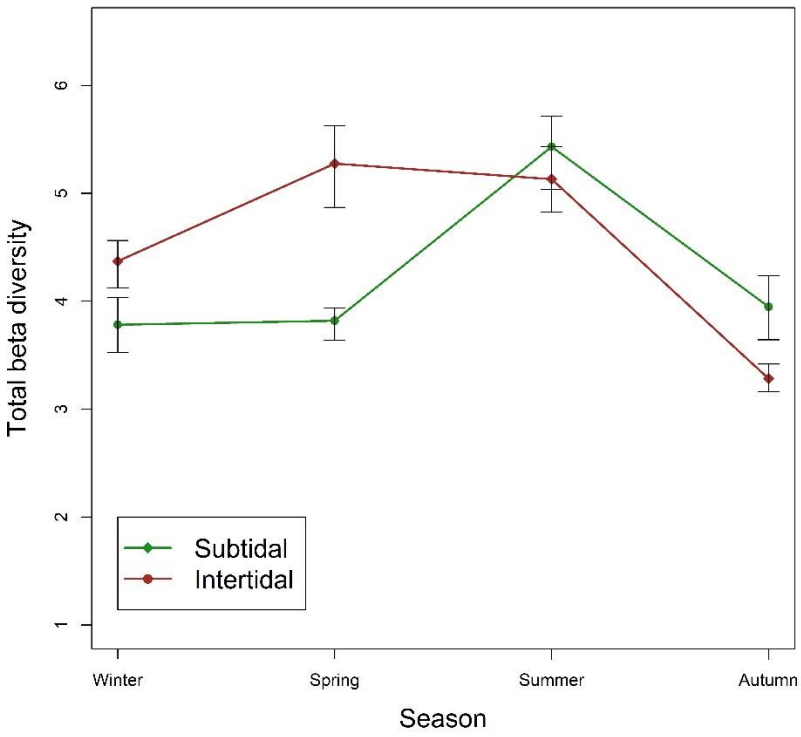


Figure 9: Seasonal variations in beta diversity across elevations. Estimates of beta follow Macron (2012) decomposition of diversity into alpha beta and gamma components. Beta diversity represents the number of effective communities within the data set (communities with no common species and of equal weights).

Beta diversity across elevations spiked significantly for the subtidal zone during the summer, with 5.43 effectively unique communities present (fig. 9). This value deviates notably from the other seasons, where the number of effective communities (beta) remained between 3.78-3.94. This suggests that during the summer, subtidal faunal community assemblage differs more so than intertidal and subtidal regions across other seasons. This result is also congruent with the severe drop in subtidal alpha diversity during the summer.

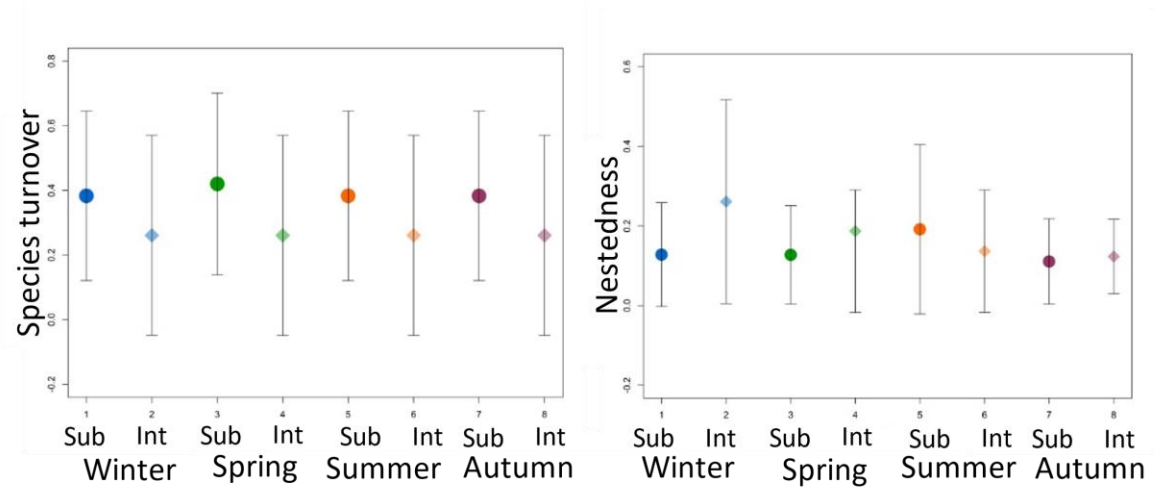


Figure 10: Decomposition of pairwise beta diversity into species turnover (a) and nestedness (b) components. Int corresponds to intertidal replicates, while sub corresponds to subtidal replicates. Points represent mean values between pairs of sites and error bars indicate 95% confidence intervals. The position of points in the plot corresponds to tidal elevation and season

Replacement in intertidal replicates is slightly less than that of subtidal, yet follows a similar pattern irrespective of season. This being said, overlapping confidence intervals between subtidal and intertidal estimates of turnover mean that differences cannot be considered as significant. Similarity, values for nestedness showed very little deviation through time and space (fig. 10). Overall, nestedness appears to be lower in the subtidal zone than intertidal zones, for example, during the winter nestedness accounted for on average 0.13 and 0.26 of total beta diversity for subtidal and intertidal zones respectively (fig 10). Spring and autumn also display slightly lowered values of nestedness throughout the subtidal zones, however, summer months show the opposite pattern. As mentioned earlier, overlapping 95% confidence intervals indicate that any differences present cannot be considered as significant.

2.4 Discussion

Spatio-temporal studies in ecology aim to understand how community compositions vary through space in time. Given that seagrass habitat is typically exposed to daily tidal fluctuations, this means that separate patches within what would be considered a single seagrass bed are likely to be experiencing highly localized environmental conditions. As described in numerous ecosystems, seagrass faunal communities in Duvauchelle bay are dominated by few common species (shrimp spp., sand flounder, and black flat-top) that occur consistently in high abundance over both sites and seasons. Moreover, the presence of highly rare species (eg. *Patiriella* sp., *Lunella smaragda*, and

Dosinia anus were only caught once) further suggests that community composition of this bay is typically comprised of several highly abundant taxa, and numerous rare species. Because of this, it is highly likely that a year of monthly sampling may not be adequate to fully describe seasonal changes.

Spatial patterns of species turnover or beta diversity is a useful metric to infer biodiversity as it captures processes of dispersal and connectivity that act toward maintaining regional biodiversity (Socolar *et al.* 2016, Whippo *et al.* 2018). Traditionally, connectivity has been difficult to quantify, especially for large-scale communities that are subject to consistent changes in abiotic conditions. Thus, patterns of beta diversity may give indication to the movement of species over space and time, which provides evidence of population and community-level connectivity through space and time (Albert *et al.* 2017, Beger *et al.* 2010, Whippo *et al.* 2018). For subtidal and intertidal regions, spatial and temporal variation in biodiversity differed substantially, particularly during the summer of 2019.

Contrary to what was hypothesized, overall species abundance and diversity was not consistently higher during the warmer months. Despite differences in sampling effort, autumnal months had considerably higher alpha diversity and individual density in comparison to summer months, which displayed unexpectedly low abundances (fig. 3, fig 7). Species replacement which implies the replacement of some species by others across seasons, elevation and bay side showed no significant differences in species composition over seasons and sites (fig. 10), which may suggests that Duvauchelle Bay does not naturally experience high rates of species replacement throughout the year. This suggests that throughout the year, species composition does not differ significantly. Juvenile fish species such as sand flounder, blennies, and banded wrasse were present throughout the year, suggesting these species do not occur in seagrasses through a particular season. Moreover, blue cod were only found during the autumn and winter months which is congruent with the timing of migration for this species. For example, studied on the seasonal distribution of blue cod show that in the Otago continental shelf, adult fish spawn during early summer, therefore, we can assume that blue cod juveniles in Duvauchelle Bay will show seasonal distribution patterns (Morrison *et al.* 2014, Robertson 1973).

Due to the nature of field sampling, it is also likely that the presence of species in single season may be the result of incomplete sampling, rather than seasonal preference. It should also be noted that abundances across all seasons are heavily driven by two taxa (shrimp, flounder). Interestingly, despite their dominance in the system, abundance for both shrimp and flounder were reduced significantly over the month of January 2002. It is also possible that seagrass associated faunal community's follows a seasonal sinusoidal pattern that peaks during intermediate seasons (spring, autumn) and drop during summer and winter in response to climatic stress. Thus, diversity and abundances would rise during intermediate seasons when the thresholds for light and temperate are less severe.

The most conventional mechanism responsible for seagrass decline under over-enrichment of nutrients is through the rapid stimulation of high-biomass algal blooms, including phytoplankton, epiphytes, and macroalgal species (Burkholder *et al.* 2007, Hauxwell and Valiela, 2004, Ralph *et al.* 2006). One possibility for reduced abundances during the warmer summer months could be that faunal assemblages were negatively impacted by the presence of problematic epiphytic algae. Inherently, seagrass beds experience fluctuations in fast-growing epiphytic algae, brought on by seasonal changes in light, temperature, and nutrients (Burkholder *et al.* 2007, Hauxwell and Valiela, 2004,). However, when in excess, such algal species form suffocating macroalgal blooms that led to rapid loss and deterioration of seagrass ecosystems through eventual processes of eutrophication (Burkholder *et al.* 2007, Hauxwell and Valiela, 2004). Photosynthetic processes for seagrasses rely heavily on light availability. Populations of *Zostera muelleri* in New Zealand and Australia have displayed reduced growth and shoot development on account of diminished clarity in the water column (Bulmer *et al.* 2016, Chartrand *et al.* 2012, Schwarz 2004). This phenomenon has become progressively common in coastal systems worldwide as a result of increased nutrient load brought on by human activities along coastal areas and freshwater ways (Broderson *et al.* 2015). Over the past several decades, seagrass meadows have experienced catastrophic deterioration, particularly in poorly flushed estuaries, coastal embayments, and lagoons where nutrients loads have remained consistently concentrated (Burkholder *et al.* 2007, Hauxwell and Valiela, 2004, Orth *et al.*, 2006). Further coastal activities such as aquaculture, motorboating, dredging, and trawling reinforce the negative impacts of large-scale algal growth by increasing turbidity and physical damage that promotes additional seagrass die-off. In combination with warmer temperatures brough on by climate change, problematic algal blooms are likely to become far more common in coastal and estuarine systems worldwide (Burkholder *et al.* 2007, Gerber *et al.* 2014). Seagrass beds in the upper intertidal zones began showing signs of stress in December (2019). Observations made during the months of January and February (2020) found large patches of seagrass to be either dead/dying or obscured by large epiphytic algae mats. Epiphytic algae were concentrated towards the upper shore but did also extend into the subtidal zone. It may also be possible that this algal growth negatively impacted faunal communities by suffocating the system, thus, resulting in reduced abundances during this time.

Seagrass beds are widely recognized as vital habitat depended on by many species. Numerous fish species have been recorded utilising food resource and refugia provided by seagrass habitat as juveniles before returning to sea (Morrison *et al.* 2014, Parsons *et al.* 2013, Unsworth *et al.* 2019). Because of this, seagrass is expected to experience changes in species composition relative to the life stages of temporary residents. Studies on the relationship between *Z. muelleri* and associated fish populations across New Zealand seagrass meadows highlighted the importance of subtidal patches as juvenile nurseries for species such as snapper, and trevally (Morrison *et al.* 2014, Parsons *et al.* 2013, Turner and Schwarz 2006). Nevertheless, the diverse nature of seagrass ecosystems illustrates that

resource and refugia values vary strongly, based on local tidal position, hydrology, and landscape settings (Morrison *et al.* 2014, Parsons *et al.* 2013). Duvauchelle bay showed a high populations of fish species across elevations, however, intertidal abundances are heavily driven by sand flounder. This species is a perfect example of a species with the capabilities to thrive all throughout the bay, though, overall abundances were typically concentrated towards the intertidal zone (Morrison *et al.* 2014). Subtidal fish communities on average display a higher species richness compared to intertidal regions, with 10 different species caught in both the seasons of spring and Autumn. This suggests that subtidal areas may provide a more stable biotic environment that is able to support larger quantities and richness of fish species (Morrison *et al.* 2014, York et al 2018).

Overall, subtidal samples contained the most diverse fish and abundant community (table 1 and 3). Species such as whitebait and Kahawai are likely to have been caught while migrating from the rivers to the sea, thus, seagrass habitat may act as a transitional area, rather than habitat. In a tidal landscape, subtidal patches provide habitat with reduced desiccation stress compared to the intertidal, thus, the rate at which species are replaced by others between tidal elevations may be driven by biotic factors. That being said, it should also be considered that sampling occurred roughly during similar tides, thus did not incorporate diurnal movements of species as the tide changed. For example, it is possible that as the tide rises, individuals found typically in the subtidal zones will migrate vertically in synchrony with the tidal cycle (Gibson 2003). While knowledge of regular vertical movement in relation to tidal variation is lacking, studies have documented estuarine species moving into tidal flats during the high tide (Gibson 2003, Morrison *et al.* 2002). Such movements of retreating into shallower waters is considered to be largely driven by reproduction, predator avoidance and feeding (Gibson 2003).

To understand ecological communities, it is imperative to investigate the processes of species distribution and abundance (Whippo *et al.* 2018). For many highly diverse and complex communities such as those in marine habitats, there is insufficient information on when, where, and why species occur over spatial and temporal scales (Aylesworth *et al.* 2017, Boström *et al.* 2006, Gastron 2000). Such processes may become much more complex in ecosystems that experience highly localised discrepancies in environmental conditions. This study further supports the high variability that is associated with seagrass faunal communities, while also highlighting what may be driving these discrepancies. For example, despite an algal bloom presumably reducing abundance during the summer, species richness was not impacted in a significant way. By providing a framework as to how this system interacts with its inhabitants over the seasons, we can better infer how they are subject to change in the face of climate change and human disturbance.

As an important foundation species, seagrasses have gained international attention for conservation to preserve biodiversity and ecosystem services. Yet, the role of spatial arrangement in conjunction with the exact processes of how these habitats support such high productivity remains poorly understood

(Boström *et al.* 2006, Whippo *et al.* 2018). To make well informed decisions about conservation and management of seagrass habitats in a changing world, it is clear that it is vital to not only understand the processes maintaining spatial mechanisms of diversity and abundance but to also consider how these mechanisms will be influenced by climate change (Boström *et al.* 2006, Gerber *et al.* 2014, Whippo *et al.* 2018).

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Chapter 3

Population parameters for native pipefish populations within seagrass beds at Duvauchelle bay

Introduction

Field and laboratory investigations have shown that natural populations of fish species are dictated by a combination of structural complexity, resource allocation, abiotic conditions, and perceived species interactions (Malavasi *et al.* 2007, Munday *et al.* 2001, Smith *et al.* 2011). To illustrate, biogenic ecosystems such as seagrasses that are characterized by high structural complexity provide the opportunity for sympatric fish species to exist with reduced predation pressure (Malavasi *et al.* 2007). Within seagrass systems, pipefish belonging to the family Syngnathidae represent an example of a taxonomic group displaying high levels of among and within habitat partitioning (Curtis and Vincent 2005, Kendrick and Hyndes 2003). Pipefish are a charismatic taxonomic group that exemplify the relationship between vegetated habitat and distinctive functional morphology (Kendrick and Hyndes 2003, Motta *et al.* 1995). The need to avoid predators is an important driver to the cryptic morphology and behaviour of pipefish which allows near perfect camouflage with leaf blades (Kendrick and Hyndes 2003, Miller 1996). However, predator avoidance must also be balanced with necessary conspicuous behaviours such as courtship and foraging (Fuller and Berglund 1996).

Due to physiology and population characteristics, pipefish are particularly susceptible decline. Although some species occasionally take part in short-term seasonal migrations, many adult species maintain a relatively small home range (Foster and Vincent, 2004). For example, species such as *Stigmatopora nigra* spend their entire life cycle within seagrass meadows, thus, habitat loss and

degradation are a main threat for persisting populations (Orth *et al.* 2006, Waycott *et al.* 2009, Vincent and Koldewey 2011). Furthermore, syngnathid species have been proven to be particularly sensitive to the negative impacts of physical and chemical degradation. For example, species *Syngnathus floridae* and *Syngnathus scovelli* that reside within seagrass beds throughout the northeast Gulf of Mexixo were significantly less abundant in regions that experienced higher levels of pollution (Livingston, 1984). Because of this in addition to their charismatic nature, pipefish have been recognised as a beneficial flagship species that can aid in determining ecosystem health of estuarine systems (Shokri *et al.* 2008, Vincent and Koldewey 2011)

In New Zealand, most pipefish species rely heavily on *Z. muelleri* as habitat. Pipefish express a role-reversed mating system, where males will take on the role of pregnancy by accepting unfertilised eggs from females (fig 1) (Braga Gonclaves *et al.*, 2016, Vincent *et al.*, 1995). Male pipefish of different species exhibit a wide diversity of brooding structures that are used to provide protection, aeration, and nutrients for developing embryos (Braga Goncalves *et al.*, 2016, Vincent *et al.*, 1995, Wilson *et al.*, 2001). Native New Zealand pipefish species originated from western Australian populations an estimated ~0.25-0.6 million years ago (Dawson, 2012). After establishing in coastal waters, the change in environmental conditions resulted in the evolution of gigantism of a species that is now classified as *Stigmatopora macropterygia* (Dawson, 2012). Populations of *S. macropterygia, Leptopnotus norae, Leptonotus elevatus, Stigmatopora nigra* and *Stigmatopora argus* have been documented present in seagrass beds throughout New Zealand, though literature regarding basic population and breeding ecology is limited. Individuals typically reside within nearshore seagrass habitats of good condition that harbour highly diverse and productive communities (Kendrick and Hyndes 2005, Smith et al 2008).

While there is limited information on habitat preference in New Zealand, we can hypothesize that species here may follow similar behaviours present in Australian populations. For example, in the temperate waters of Victoria, Australia, the pipefish species *Stimatopora nigra has* been found in have been found to congregate around seagrass edges where prey types such as planktonic copepods and small crustaceans are more plentiful (Kendrick and Hyndes 2005, Jackson *et al.*, 2001, Smith *et al.* 2008, Tanner 2005). Therefore, we may expect that *S. nigra* populations in New Zealand prefer seagrass edges, though, due to the change in environmental conditions, more research would be required to determine this.

Methodology

Study site

Duvauchelle bay, located at the head of the Akaroa harbour possesses large expanses of lush seagrass habitat. As this location is fairly isolated from large-scale human development, healthy beds of *Z. muelleri* here are present in abundance all year round, therefore is prime habitat for native pipefish species associated with seagrass habitat. Seagrass ecosystems experience consistent changes in abiotic conditions, thus, for this study the bay was split by elevation and bay side to further examine more localised effects of distribution. Elevations include intertidal and subtidal habitat, whereas, bay side correlates to the left or right side of the bay (fig. 11)

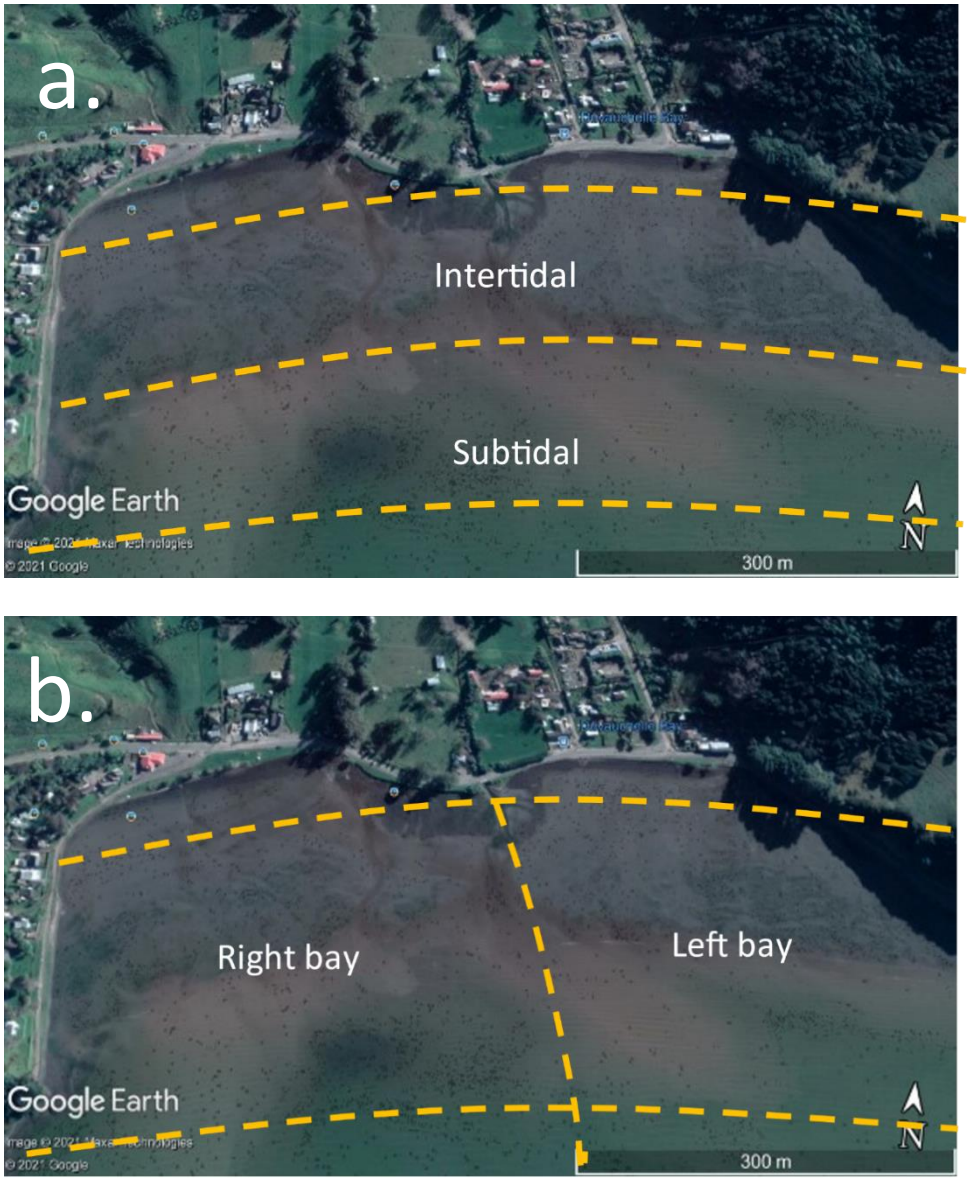

Figure 11: Aerial shot of Duvauchelle bay. For analyses the bay was sectioned into zones that represented elevation (a. subtidal, intertidal) and bay position (b. right, left) to examine localized changes in community composition over the seasons.

Seine sampling

Sampling commenced at Duvauchelle just after high tide while the seagrass is submerged. Seagrass beds and bare patches were sampled using a modified 1.2m x 1.2m seine net mounted on a PVC frame that is hand-hauled, parallel to the shore in a series of non-overlapping pulls with tows ranging between 3-15m (fig 4). The net has a mesh of 0.05mm to avoid smaller organisms (eg juvenile fish spp.) being excluded. At the start and end of each tow, seagrass density was visually scored and categorized as either sparse, patchy, dense, or bare substrate (fig. 1) Coordinates (lat/long) and time were recorded at the start and end of each tow on a Garmin GPS. At the end of each tow, the net was sorted through and all living organisms are identified, counted, and recorded. To keep track of different developmental stages, some species were categorized into rough sizes of >10mm, 10-20mm, 20-50mm, and >50mm using a 0-50mm scale on the frame of the net. Any organism in the field that could not be identified or is of interest was photographed for later identification. The percentage of live seagrass roots in the net were visually estimated to give an indication whether seagrass was being uprooted by the net. From the experience of myself and my sampling team, we can conclude this method causes minimal impact (Thomsen et al 2020).

Processing sygnathids

Any sygnathids caught via seine net were transferred into buckets and taken to shore to be anesthetized with 3 drops of clove oil in 500ml of seawater water. Individuals are identified to the species level as either *Stigmatopora nigra* or *Leptonotus elevatus* based on morphological traits (Roberts et al 2015). Individuals are measured to the nearest mm and photographed. Photographing allows us to investigate morphological changes throughout the year by providing a visual record of pipefish caught for accurate length measurements. The sex of each is determined by the presence of a brood pouch located on the ventral side of males (fig. 13). Full body length for brooding *S. nigra* males ranges between approximately 70-160mm, thus, *S. nigra* individuals over 70mm length lacking a caudal pouch were recorded as female (Dawson 1982, Howard and Koehn 1985). Reproductive females were identified by the presence of a mating ornament, as seen in figure 13. Individuals under this size were categorized as juveniles considering that immature males have yet to fully develop a brooding pouch and cannot be identified confidently (Dawson 1982, Howard and Koehn 1985). Similarly, *L. elevatus* juveniles were determined later based on the smallest male (60cm), thus any individual caught under 60cm were classified as juvenile. Moreover, caught *L. elevatus* individuals longer than 60cm and lacking a pouch caught were considered females. The pregnancy status of males is visually determined by the presence of eggs in the brood pouch.

Individuals caught in Duvauchelle that were considered adult were also marked with Visible Implant Elastomer Fluorescent Tags provided by “Northwest Marine Technology”. As pipefish are small and

narrow, traditional tagging of individuals may be inappropriate and cause unnecessary bodily harm. Visible implant tags provide a more appropriate method and has proved beneficial in other long-term population studies involving small fish species (Frommen *et al.*, 2015, Dunn, and O'Brien 2018). Prior to tagging, individuals were fully anesthetised coloured plastic tags were injected just below the skin in a series of coloured lines on either side of the abdomen (fig. 13) This allowed different individuals to have a unique marker thus, providing accurate identification at the individual level during later processing (fig 5). Masonjones et al 2010, Rose et al 2013). Tagging pipefish allowed us to investigate whether the same individuals remain in the area throughout the study period. After individuals recovered, they were gently released back into subtidal seagrass habitat.

Figure 12: Adult Stigmatopora nigra individuals captured via seining in Duvauchelle bay. The female (A) is easily identified by the presence of horizontal bands and enlargement of the abdominal cavity. The male (B) can be identified by the presence of a sub caudal brooding structure and the pregnancy status can be identified by the presence of eggs in said structure.

Figure 13: Photos showing VIE tags on adult pipefish individuals as represented by red lines that are injected just beneath the skin. Each individual is tagged with a unique combination of coloured lines that allow us to identify if individuals are recaptured. On the right (B) is a female L. elevatus which was identified by the absence of a ventral pouch, while on the left is a male S. nigra.

Data analyses

All data analysis was performed using the programme Rstudio. Individual counts per month were pooled seasonally to examine reproductive timing and growth throughout the year. Due to unequal variances, seasonal interspecific variations for juvenile and adult cosnigunts and size were analysed using non-parametric Wilcoxon Rank Sum tests. Frequency of body lengths for juveniles, males, and females were generated as plotted with the minimum age of maturity for each species. Summary statistics for body length (mm) and abundance were generated for both species to examine changes in life history traits. This allowed us to speculate the rough timing of average growth throughout the population. For example, is a particular season only shows lengths for mature individuals, we can assume that juveniles are not present in seagrass habitat during this time.

Sex ratios for each species were calculated and compared between tidal elevations and seasons using the R function binom.test(), as recommended for datasets with small samples. This function tested if populations of males and females differs significantly from a 1:1 sex ratio over the seasons and

provided the alternative hypothesis, 95% confidence intervals and the calculated probability of success. The proportion for juveniles, males, and females for both species per season was calculated to further examine changes potentially related to breeding time and age. Interspecific variations for sex-ratio relative to season and elevation were tested using non-parametric Wilcoxon Rank Sum tests.

As assumptions of normality are not met, differences in total pipefish counts were tested using Poisson generalised linear models relative to season, bay-side, elevation, and seagrass cover (fig.11 and 12). Models were generated for pipefish communities as a whole and for *S. nigra* and *L. elevatus* species independently. In doing so, we can infer the response of pipefish abundances as a whole, in addition to examining patterns shown by specific species to the multiple abiotic conditions included. The benefit of using Poisson GLMs in this case lies in the way in which error is handled relative to data type. Poisson GLMs are suitable for any ecological data that is comprised of count data.

Results

Across 12 sampling events that spanned November 2019 – September 2020, a total of 83 pipefish individuals were caught. Of those caught, 63 were of the species *Stigmatopora nigra*, while 20 were of the species *Leptonotus elevatus*. Of the 31 adult individuals that were tagged, none were recaptured in succeeding sampling events. Generalized linear models found no significant differences in total abundances of pipefish across season (P=0.901), tidal elevation (P=0.721), and seagrass cover (P=0.789). Similarly, no significant effects were detected when analysing S. *nigra* and *L. elevatus* as independent species across season, tidal elevation, and cover.

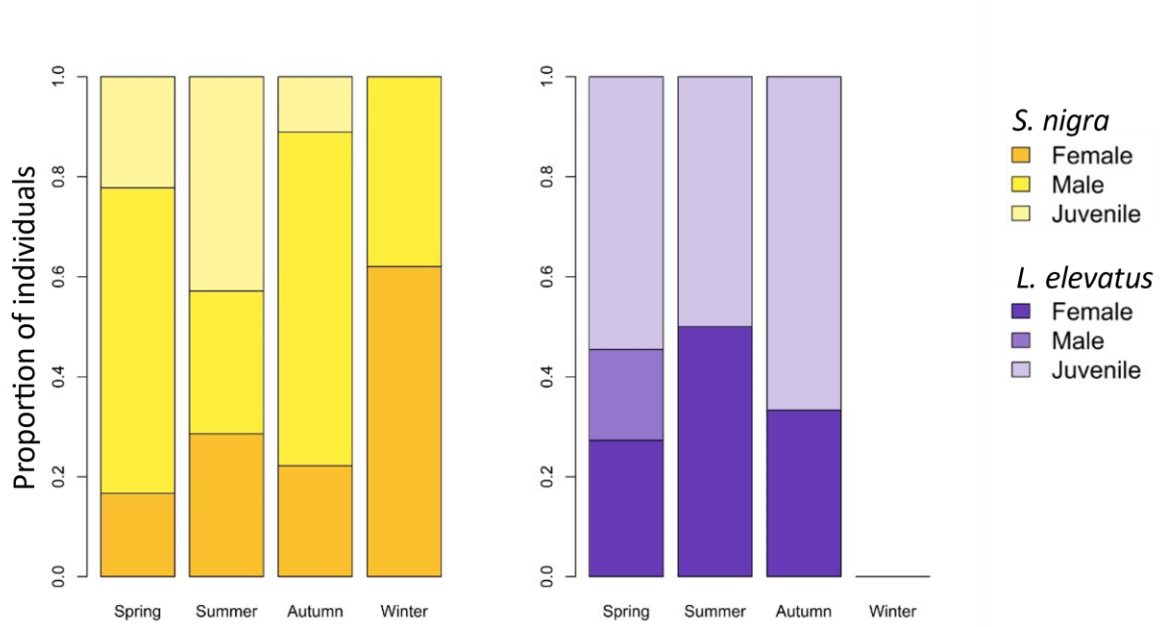

Figure 14: Proportions of females, males, and juveniles for Stigmatopora nigra (a) and Leptonotus elevatus individuals caught over seasons. For S. nigra and L. elevates respectively, caught individuals under 60mm and 70mm were considered juvenile.

S. nigra populations across seasons on average, were comprised mostly of males and females that ranged from 70-158mm in body length (fig. 15, 16, and 17). Overall, body length differed significantly in range over seasons (Wilcoxon, $P<0.01$), whereas overall adult male and female length fell within similar ranges (Wilcoxon, $P<0.01$, (fig. 16 and 17). *S. nigra* juveniles were present throughout the seasons of spring to autumn, with the youngest individuals caught during the spring (fig. 16). On average, body length for *S. nigra* individuals was highest in the spring and lowest in the summer. Throughout summer and autumn, average body length for *S. nigra* individuals ranged just below the minimum age of maturity. Winter populations were completely characterized by adult pipefish, with a higher proportion of females to males' present (fig. 15 and 16).

In contrast, *L. elevatus* populations were dominated by juveniles throughout the year, with exception to winter where no individuals were caught (fig. 15). Adult males remained rare throughout the year and were only found during a single month of spring (November, 2019) Overall, body length ranged from 51-103mm, with both the smallest and largest individuals found during the spring. Average body length increased slightly over the seasons, with summer and autumn months comprised completely of adults. Unlike S.nigra, body length differed significantly between males and females. Nevertheless, very few *L. elevatus* individuals were caught during the entirety of this study, therefore, more sampling is required to ensure this result.

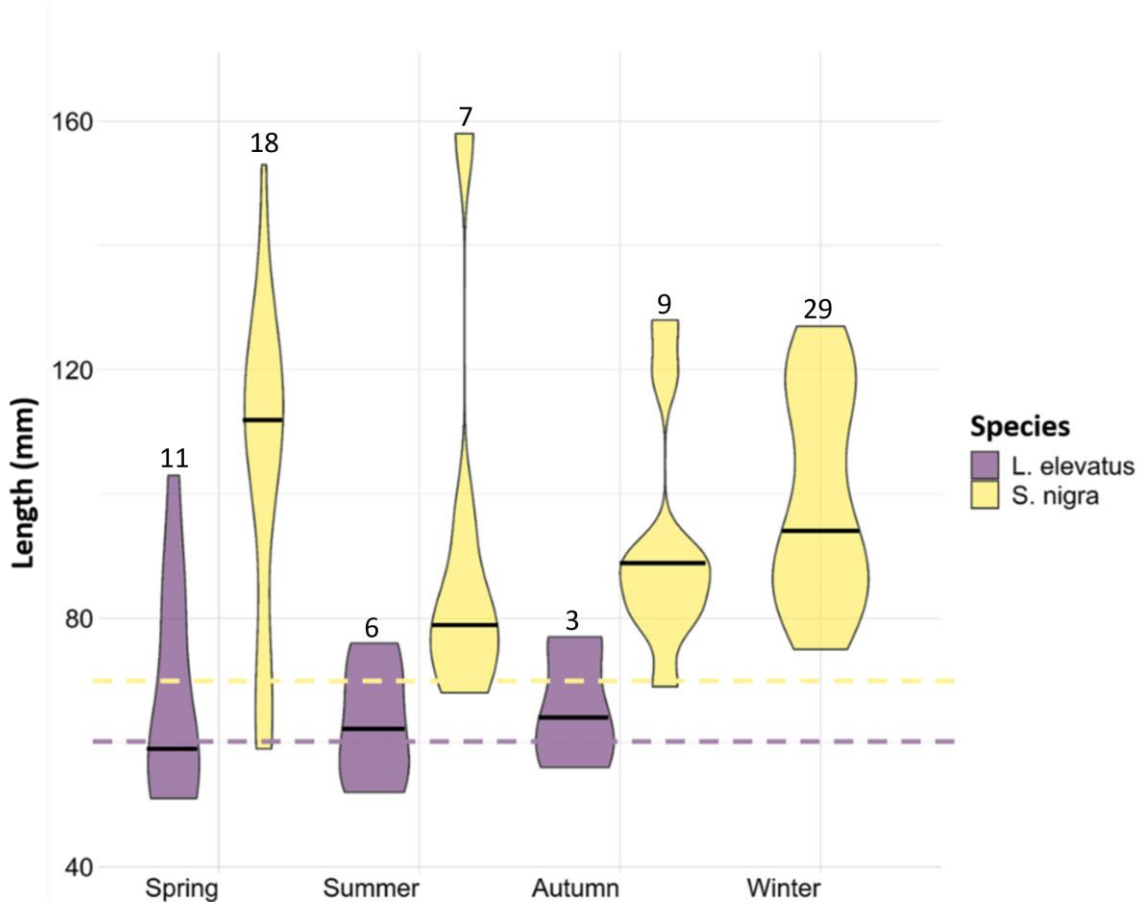

Figure 15: Violin plot displaying variation in individual length (mm) of all individuals for species Stigmatopora nigra and Leptonotus elevatus across seasons. Means for each violin is demonstrated by the black line, while the minimum size of maturity of each species is represented by dotted lines. Total individuals caught are displayed above.

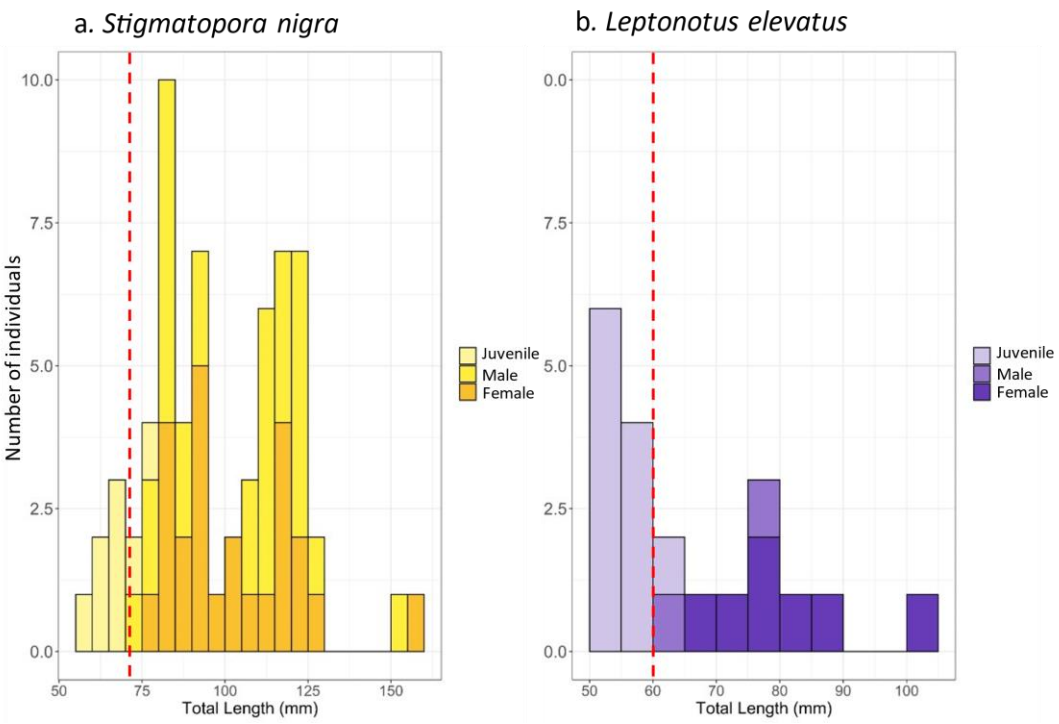

Figure 17: Frequency distribution for total body length (mm) of females, males and juveniles caught between November 2019 and September 2020. Minimum length for adult maturity is represented by the red dotted line. Minimum length for S. nigra and L. elevatus is 70cm and 60 cm respectively.

For S. nigra adults, male-to-female ratios varied across time space (Fig. 18). Across tidal elevations, the average ratio for males is higher in subtidal zone (Wilcoxon, $P<0.01$), however, confidence intervals for the intertidal zone indicate that the differences seen may fluctuate considerably. As for seasonal differences (Wilcoxon, $P<0.01$), winter displayed the highest proportion of females to males, whereas spring and Autumn show a male-biased sex ratio, however, these may be subject to change (fig. 18). In contrast, males of the species *L. elevatus* were only found in subtidal replicates during the spring. No male individuals of the species *L. elevatus* were caught during the winter, summer, and autumn, moreover only females were caught throughout the intertidal zone. As stated previously, very few individuals of this species were caught, therefore, results may be heavily influenced by the presence of one or a few females.

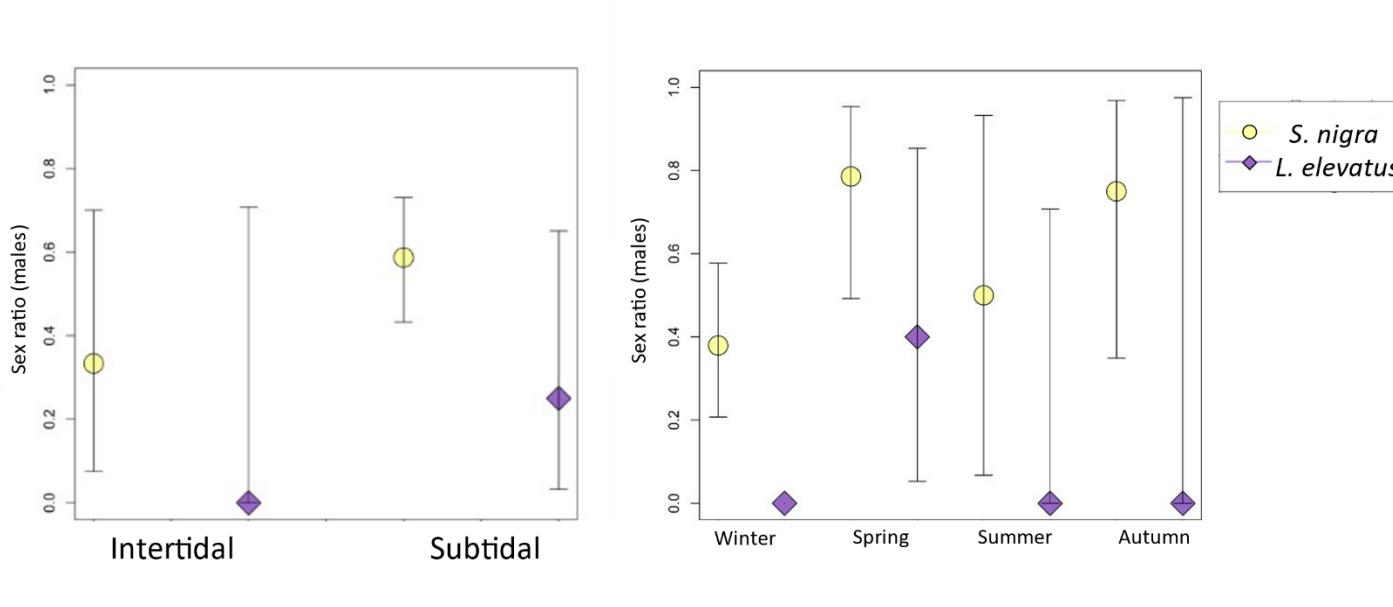

Figure 18: Mean Sex-ratios as proportion of adult males to total adults and estimated 95% confidence intervals for S. nigra and L. elevatus across tidal elevations and seasons.

Overall, patchy seagrass supported the highest numbers and were mainly dominated by *L. elevatus* juveniles and *S. nigra* adults. For both species, average counts between cover types were found to be vastly different, despite having no statistically significant differences (GLM, P=0.789). In comparison, other cover types were found to support much lower average abundances, with only 3 *S. nigra* individuals present in bare replicates. Populations of *S. nigra* were more abundant overall across all levels of cover.

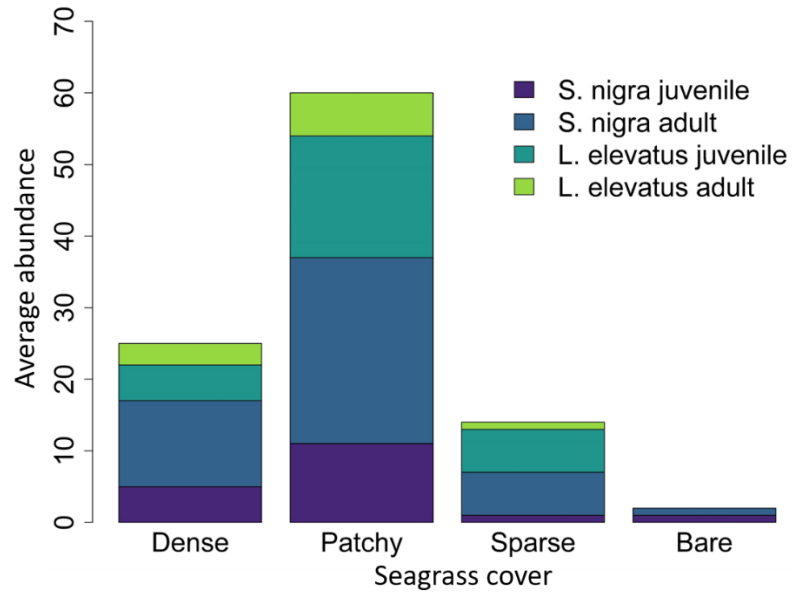

Figure 19: Average abundance for adult and juveniles of species Stigmatopora nigra and Leptonotus elevatus over categories of seagrass cover.

Discussion

Throughout the seasons, *S. nigra* and *L. elevatus* populations displayed variation in abundance, body length, and sex ratio. While both species display increased juvenile activity during the spring months, ranges for average body length and abundance were markedly higher for *S. nigra* juveniles. Furthermore, *L. elevatus* populations held higher numbers juveniles throughout the year, whereas, S. nigra juveniles were mainly concentrated during the spring. This suggests that these two species may exhibit growth differences growth throughout the year, for example, *L. elevatus* juveniles may mature at a slower rate than *S. nigra* juveniles.

Determining breeding seasons of taxa has traditionally been difficult as many studies do not or are unable to successfully determine reproductive states and to date, few studies have included pregnancy status. Similar to many life traits seen in pipefish species, seasonal breeding times are strongly correlated with latitude, however this has been found to vary within species ranges (Foster and Vincent, 2004, Rose et al, 2019). Thus far, there are no studies exploring the general ecology of native New Zealand pipefish species. Research has established an ecological framework for S. nigra populations associated with Australian seagrass beds, however, given the highly variable nature of seagrass ecosystems over time, we cannot assume that New Zealand population will follow the same patterns (Smith *et al.* 2009, Vincent *et al.* 1995). For example, ocean and air temperature on average is much lower in New Zealand, therefore, we can assume that New Zealand populations may have adjusted breeding seasons to better suit local conditions (Monteiro *et al.* 2001). Reproductive females were only found during December 2019, whereas pregnant males were caught only during November 2019, and September in 2020. Because of this we can assume that adults become reproductively active during the early spring and copulation and egg laying commence before the summer months. This would explain why pregnant males were only present during the spring. Spring months for both species were also characterized by a wider range of sizes as very large adults in addition with the smallest juveniles were present.

Pipefish abundances relative to cover illustrated here further exemplifies the benefit of utilising seagrass edges as foraging and reproductive habitat. Throughout this study, pipefish species followed a clear habitat bias towards patchy seagrass. These results are congruent with Australian studies, where *S. nigra* individuals were found to be much more abundant toward the seaward side of the seagrass edge, where cover is typically patchier than dense, suggesting that patchy seagrass or seagrass edges must provide an advantage over other microhabitats. (Smith *et al.* 2009). Moreover, seagrass edges are plentiful in small crustaceans and planktonic copepods of which are the main food source of pipefish, which suggests that *S. nigra* and *L. elevatus* individuals from Duvauchelle bay may utilise patchy or edge habitat for its greater resource availability (Kendrick and Hyndes 2005,

Smith *et al.* 2009, Tanner 2005). As prey availability changes over time, be that diurnal or seasonal, we can expect *S. nigra* and *L. elevatus* distributions will change accordingly.

Pipefish are widely recognised as important indicators of habitat health and connectivity. Their reliance on a healthy and stable seagrass habitat makes them a perfect species to infer ecosystem health. This metric will only be useful if sites sampled have had known historical populations of pipefish, and thus can be used for comparison. For example, if pipefish in Duvauchelle bay disappear, we can assume that this environment is experiencing a concerning degree of human disturbance in terms of maintaining biodiversity.

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Chapter 3

Assessing spatial variation of associated faunal communities throughout the South Island.

3.1 INTRODUCTION

Seagrass beds are a major component of estuarine ecosystems and are crucial in sustaining a diverse community of native flora and fauna (Lundquist *et al.* 2018, Morrison *et al.* 2014, Turner and Schwarz 2006). The exact composition and abundance of species present can vary greatly over space based on local biotic conditions, however, the typical *Z. muelleri* community is comprised of epiphytes, micro sessile and mobile epifauna, infauna, and larger epibenthic species (Boström *et al.* 2006, Turner and Schwarz 2006). In addition to refuge and resources, seagrasses are also ideal nursey ground for many marine and estuarine species (Morrison *et al.* 2014). Numerous commercial fishery species in New Zealand utilise rely on seagrass habitat as juveniles, which further suggests that adults out at sea rely on healthy seagrass to maintain population numbers (Grech *et al.* 2012, Morrison *et al.* 2014, Orth *et al.* 2006)

In New Zealand, seagrass populations are represented by the single species *Zostera muelleri*, which grows predominantly throughout the country's soft sediment estuaries and sheltered harbours (Turner and Schwarz 2006). As an interface between land and sea, estuaries experience constant fluctuations in a plethora of biotic conditions to which the nature of and extent is largely determined by the combined influences of underlying geology, size and incline of the catchment, freshwater inputs, and tidal cycles (Hume 2007). *Z. muelleri* is fully adapted to brackish estuarine conditions (Anderson *et*

al. 2019, Turner and Schwarz 2006). This allows *Z. muelleri* in New Zealand to occur across a latitudinal gradient, from Parengarenga Harbour (-34.524, 172.939) near the top of the North Island to Stewart Island (-47.010, 167.827), the known southern limit for seagrass distribution worldwide (Inglis 2003).

Latitudinal patterns of biodiversity for marine communities have received renewed interest in the wake of climate change, as variations in temperature are often closely correlated with latitude (Chaudhary *et al.* 2016). Variations in latitudinal diversity gradients for seagrass faunal communities are largely driven by local abiotic and biotic factors such tidal regime, wave exposure, temperature, vegetation structure, availability of resources, and more. In addition, large scale variation in community assemblages across different localities may also be the response of direct and/or indirect human disturbance (York *et al.* 2018, Turner and Schwarz 2006, Virnstein *et al.* 1984). The general understanding is that latitudinal diversity follows a unimodal pattern centred at the equator, where species richness is highest towards the equator and decreases as latitudes increases. However, recent evidence has shown that marine communities are switching to a bimodal structure, where richness drops near the equator in response to increased temperatures brought on by global warming (Chaudhary *et al.* 2016, Menegotto and Rangel 2018).

In 1999, Short *et al.* predicted that seagrass species would experience significant shifts in productivity, distribution, and community composition as a result of temperature stress. Present-day evidence has found that many species are unable to acclimate at the rate conditions are currently changing, for example, populations of *Posidonia oceanica* in the Mediterranean has suffered severe mortality rates and are predicted to experience further losses with future ocean warming (Marba and Duarte 2010, Jorda`*et al.* 2012). In addition, temperate seagrasses in western Australia have begun retracting their ranges in response to warmer temperatures (Hyndes *et al.* 2016). Distributional shifts such as these are expected to have significant impacts on associated faunal communities, particularly those in equatorial regions (Collier *et al.* 2011, Unsworth *et al.* 2019). Sites within Shark Bay in western Australia also experienced catastrophic dieback (>90%) in response to an unprecedented marine heat wave in 2010/11, an event believed to have been induced by global warming (Thomson *et al.* 2015). As a predominant habitat forming species, the loss of seagrass within this induced a myriad of impacts on the faunal community, including temporary or permanent range shifts towards the poles in megafauna, and mass mortality in fish and invertebrates (Pearce and Feng 2013, Smale and Wernberg 2013, Thomson *et al.* 2015)

Worldwide, human activity has had a profound impact on seagrass ecosystems (Iacarella *et al.* 2018, Orth *et al.* 2006) . As an estuarine associated system, seagrasses are particularly susceptible to undesirable effects of climate change and land use (Turner and Shwarz 2006). Seagrass biomass and production is restricted primarily by photosynthetic limiting parameters such as water clarity, depth,

and nutrients (Ismail 2001, Turner and Schwarz 2006). Seagrass beds often occur at the base of the catchment, where freshwater enters the sea. This means that any matter that enter the rivers from adjacent lands will eventually empty out into the estuary (Gibbs *et al.* 2012, Turner and Schwarz 2006). Although estuaries inherently experience deposits of terrestrial nutrients and organic from freshwater inputs the intensification of land use by humans has greatly increased estuarine sedimentation rates worldwide (Inglis 2003, Schubel 1984, Swales *et al.* 2002). In addition,

When suspended sediments greatly exceed natural thresholds, this may induce the smothering and eventual mortality of seagrass beds and their associated biotic communities (Duarte *et al.* 1997, Hirst *et al.* 2017, Kennedy *et al.* 2010). Naturally, seagrasses are adapted to tolerate moderate threshold of sediment build-up, however, this is dependent on the volume and properties of the sediment entering the system, in addition, to the morphology and species of seagrass involved (Turner and Schwarz 2006). For example, patches of *Z. muelleri* were found to display tolerance to experimental sediment burial with larger patches exhibiting higher resilience and recovery than smaller patches (Soerensen 2020). In contrast, species such as *Cymodocea nodosa, Cymodocea rotundata, Halophila olavis, Posidonia oceanica, Syringodium filiforme, Zostera marina* and *Zostera noltii* were found to experience 100% mortality when exposed to experimental sediment burials (Cabaço *et al.* 2005, Duarte *et al.* 1997, Mills and Fonseco 2003).

Over the past few decades, cases of mass dieback and fragmentation in seagrass habitat have been attributed anthropogenic activities and climate change (Gavish *et al.* 2019, Grech *et al.* 2012, Erftemeijer and Roy 2007, Seddon *et al.* 2000, Unsworth *et al.* 2018). Documented cases of large-scale seagrass dieback in New Zealand have historically been few and far between (Turner and Schwarz 2006). Early personal accounts suggest that seagrass beds were once more widespread, with notable decline first being reported in the 1920s/1930s with extensive and unregulated losses continued into throughout the mid and late 1900s. Considering the paucity of known historic distributions, overall losses are expected to be much greater than originally documented (Inglis 2003, Turner and Schwarz 2006). *Z. muelleri* supports a diverse range of species and vital ecological processes within many of New Zealand's estuaries (Battley *et al.* 2011, Dromgoole 1983). While the relationship between *Z. muelleri* decline and its associated faunal communities remains understudied, evidence of diversity loss and community shifts toward more and bioturbating species have been well documented as a consequence of seagrass loss (Githaiga *et al.* 2019, Lundquist *et al.* 2018, Reed and Hovel 2006). This suggests that continuous loss of *Z. muelleri* in New Zealand has the potential to significantly alter the structure of native faunal populations and cause biotic homogenization.

Marine habitats are especially dictated by infinite biological pathways that interconnect in a series of dynamic environmental gradients and determine how species and individuals exist over space (Gavish

et al. 2019, Simard *et al.* 2012). In many studies, seagrass habitats are considered a single entity, yet beds naturally exhibit a wide range of local variation, particularly in the extent of vegetive cover. For example, the occurrence of benthic invertebrates in seagrass has been found to be highly correlated to gradients for vegetative cover (Battley *et al.* 2011, van Houte-Howes *et al.* 2004) Thus, it is imperative to incorporate multiple levels of abiotic and biotic organisation when inferring spatial patterns of assemblage within and between communities, especially in the face of anthropogenic change.

The goal of this study is to evaluate spatial variation of *Z. muelleri* faunal community assemblage and structure in response local treatments of latitude, site and vegetative cover within South Island estuaries. Firstly, we hypothesize that seagrass community assemblage across sites and regions will have similar species present though as distance between sites increases, similarity between communities will decrease, in addition, diversity will increase as latitude decreases. Secondly, seagrass cover density will have an influence on epifaunal diversity on a local scale, and that seagrass patches will have higher diversity and abundances compared to unvegetated habitat. Thirdly, we hypothesize that fish communities will exhibit high spatial variation across cover types at both local and regional scales.

3.2 METHODS

3.2.1 Study design and site choice

To explore spatial variation, seagrass communities were sampled at nine sites across the wider Nelson/Marlborough, Canterbury, and Otago areas (fig. 1). This study follows a hierarchical nested design (fig.2) to test the variability of faunal assemblage across treatments of seagrass cover nested within site, nested within three fixed regions. For this, three estuaries were sampled per region.

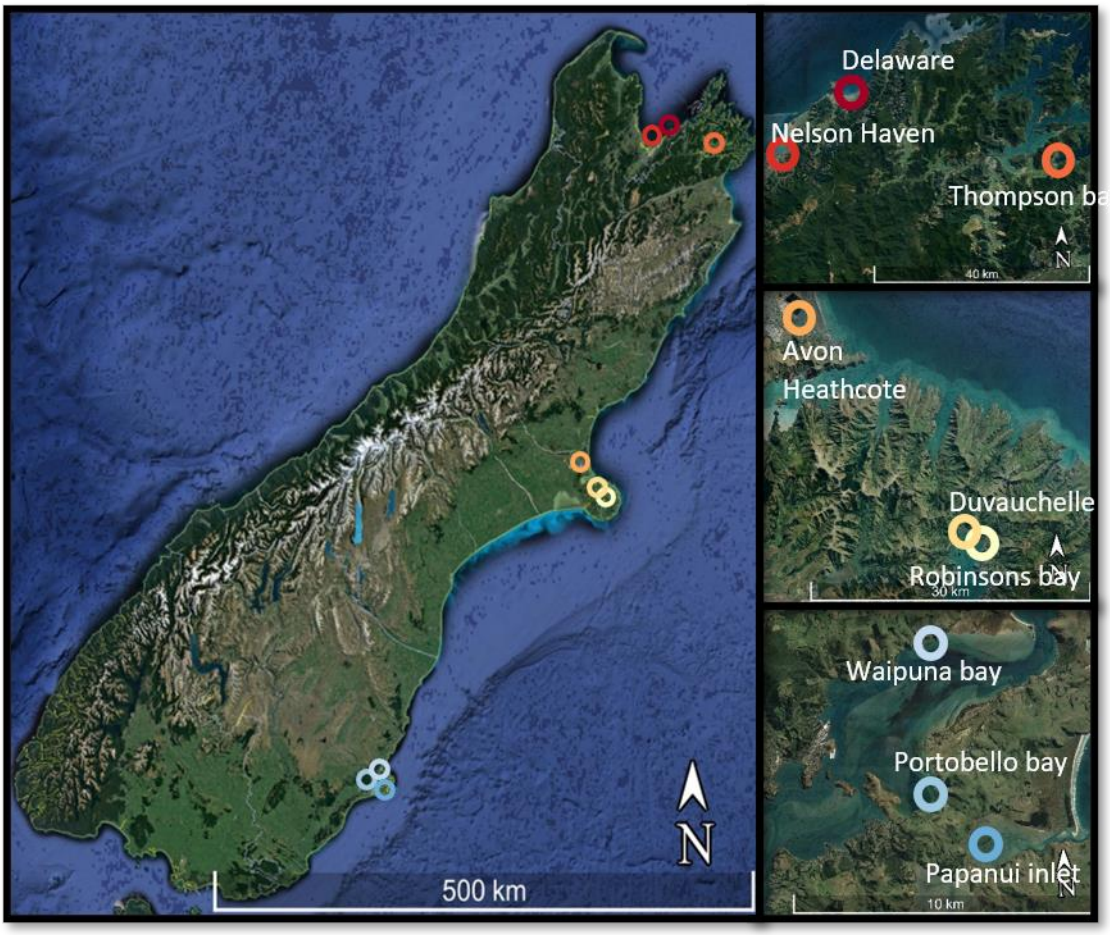

Figure 19: Position of nine sampling sites across the North, Central, South regions in the South Island of New Zealand. Colours represent geographical positioning of sites where red is furthest north and dark blue is furthest south.

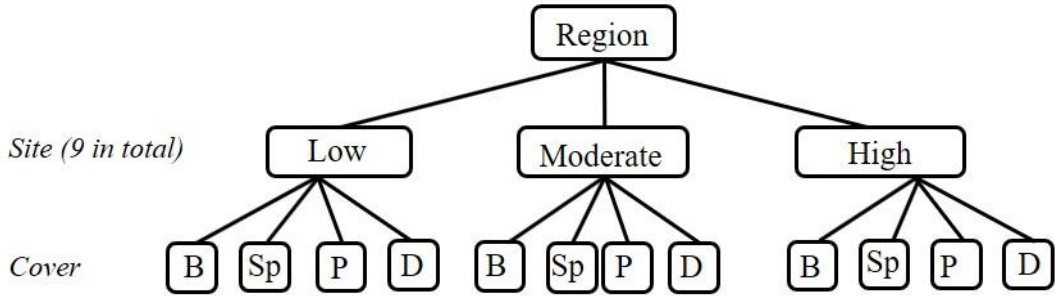

Figure 20: Hierarchical sampling design where treatments for seagrass cover are nested within each treatment of human disturbance, nested within each of the three fixed regions. Measures for cover include sparse (Sp), patchy (P), and dense (D) with bare sediment (B) as a control variable. A total of nine sites were chosen, with three sites per region.

Prior to sampling in the field, potential sites were examined using information provided by available literature. Appropriate sites were initially determined by location, size, and condition of *Z. muelleri* beds within the chosen regions, though, it should be noted that most available literature on current and

historical distribution of seagrass habitat in the South Island is sparse and/or outdated. To test the effects of vegetative cover versus bare cover on community assemblage, sites were required to have seagrass beds that included different densities of shoot cover, in addition to bare sediment habitat. Treatments for seagrass cover were categorized into sparse, patchy, and dense with bare sediment replicates acting as a control.

Final decisions for sites were selected in the field, based on accessibility to seagrass from the shore, size and condition of beds, tidal regime, and human activity (low, moderate, high) so that each region has a representation for each level of disturbance (three per region, nine in total). Extent and condition of seagrass patches in the field was determined based on visual observations of cover, colour, presence of dead seagrass and presence of epiphytes. Patches that were predominantly dead or smothered by epiphytes were emitted.

3.2.2 Study sites

Nelson/Marlborough.

Thompson's bay (-41.2689° S, 173.9113° E). is a long but narrow, sheltered inlet situated at the land base of Queen Charlotte sound (Gillispie *et al.* 2012) It is the second most inland bay within the Groves Arm harbour, adjacent to Okiwa bay which is characterized by areas dominated by salt marshes. Forested hillsides slope steeply towards the shore where the seabed descends gently before dropping off steeply into the Groves Arm harbour (Gillispie *et al.* 2012, Stevens 2018). Freshwater enters directly into the bay from the small Te awa steam that flows down from the hills and through the small settlement of Anakiwa before entering the bay. Healthy seagrass patches of differing density were present throughout the bay, growing along the tide line and extending out into the harbour (Gillispie *et al.* 2012, Stevens 2018).

Delaware inlet (-41.1471° S, 173.4657° E) is a moderately sized (336ha), tidal lagoon type estuary, located at the mouth of the Wakapuaka River, approximately 19km Northeast of Nelson City (Stevens and Robertson 2017, Šunde *et al.* 2017). This estuary is fed mainly by the Wakapuaka River and a number of small creeks that flow down from neighbouring agricultural land. As the inlet slowly drained, healthy seagrass patches were revealed, roughly 100-150 meters from the shore (Gillispie 2008, Stevens and Robertson 2017). Patches were concentrated around several fast-flowing channels that remained permanently submerged during low tide. Small patches of subtidal seagrass were present within these channels (Stevens and Robertson 2017, Šunde *et al.* 2017).

Nelson Haven estuary (-41.2425° S, 173.2987° E) is an extensive (1242ha), shallow and well flushed tidal lagoon type estuary located directly in front of Nelson city and dominated by mudflats (Stevens and Robertson 2017). A 13km man made boulder bank separates the estuary from the wider Tasman bay area while the Matai, Nelsons largest river runs through the estuary toward two tidal openings

divided by Haulashore Island. Due to its shallow topography and wide tidal range of 1.2-4.2m, large expanses of mudflat are left exposed during low tide (Stevens and Robertson 2017, Gillispie 2008) . Seagrass here were present in large patches along the mid to low intertidal zones, ranging from 150-300 meters offshore. Subtidal seagrass patches were present within deeper channels and catchment areas that remain submerged during low tide (Stevens and Robertson 2017, Gillispie 2008).

Canterbury

Duvauchelle bay (-43.7504° S, 172.9334° E) is a large inland estuary situated in the Akaroa harbour, ranging approximately 800m wide and extending roughly 1km towards the harbour (Hart *et al.* 2019, Richie 2015, Thomsen *et al.* 2020). A freshwater inlet flows centrally through the shore, creating a natural barrier between the two sides of the bay as seagrass appears to not grow around this inlet. During low tide, a large portion of the bay becomes exposed due to the flat shoreline which only descends 1.5 meters (Hart *et al.* 2019, Ritchie *et al.* 2005). The seabed is dominated by silty mudflats which facilitate healthy, well-established seagrass beds that exist at differing densities along the intertidal and subtidal gradients. Sparse patches are present ~150-200 meters from the back shore and increases in density along the tidal gradient (Thomsen *et al.* 2020).

Robinson's bay (-43.7576° S, 172.9693° E) is a smaller estuary that neighbours Duvauchelle and fed by two small creeks that run through neighbouring agricultural land (Hart *et al.* 2019, Ritchie *et al.* 2005). This bay has a 400m wide intertidal zone that descends at into the harbour at a steeper rate than Duvauchelle. Because of this, seagrass beds do not extend far back and are mainly present in the mid and upper intertidal zones (Hart *et al.* 2019, Ritchie *et al.* 2005). These zones consist of mudflat, rocky shore, and dead shell habitat where seagrass grows in dense and sparse patches throughout. Majority of seagrass patches within the main tidal zones were of patchy and sparse densities with dense patches occurring only along the eastern fringes of the bay (Hart *et al.* 2019, Ritchie *et al.* 2005).

The Avon-Heathcote estuary (-43.5455° S, 172.7293° E) is nestled within the city of Christchurch and spans 8.8km^2. Freshwater inputs flow from the Avon river as well as the Heathcote river and create a variable salinity gradient throughout the estuary, ranging from 8-15psu near the river mouth to 22-34psu near the estuary mouth (Bolton-Richie and Main 2005, Gibson and Marsden 2016). Its proximity to urban development has led to this estuary experiencing a high degree of human induced disturbance from as polluted runoff, inputs of sewage and construction in the surrounding area (Gibson and Marsden 2016). Seagrass was present in large patches across the estuary and concentrated across the intertidal zone. Patches along the intertidal zone were mostly sparse and patchy with few dense patches only present in deeper waters (Gibson and Marsden 2016, Thomsen *et al.* 2020).

Otago Peninsula

Waipuna bay (-45.7881, 170.6731) is a large, flat, coastal embayment, approximately 500m across and extending a further 500m into the Otago harbour. This site is located 8.7km away from the closest largescale urban development (Port Chalmers) and 22.4km away from the city of Dunedin (Single *et al.* 2010). Freshwater inputs are restricted to the single creek (Abernethys Creek) that trickles down from the hills, hence the water gradient of this bay is dominated by oceanic waters (Single *et al.* 2010). Seagrass was present in large expanses of dense patches all throughout bay, starting at the high intertidal zone, and extending relatively far into deeper subtidal water. Seagrass fronds within deep subtidal patches of this site were observed as being significantly longer than seagrass seen at other sites.

Papanui inlet (-45.8459, 170.6926) is a flat, vast inlet that spans approximately 2km across and extending a further 4km into Wickliffe Bay on the pacific coast of the Otago Peninsula (Mills and Berlenbusch 2008). Majority of the surrounding area is comprised of large expanses of sloping agricultural and industrial land. Freshwater inputs into the upper tidal area are restricted to a handful of waterways that flow down from neighbouring agricultural land. Further down, another small stream enters the estuary from the Okia Flat wetlands (Mills and Berlenbusch 2008). At low tide, the inlet drains slowly leaving wide expanses of shoreline exposed. Sparse and patchy seagrass were present throughout the inlet; however, patches of dense seagrass were limited. Patches of dead or dying seagrass were also present, particularly along the northeaster foreshore. Within the mid and subtidal zones seagrass was covered by thick mats of the bloom-forming macroalgae, *Ulva Lactuca* making it very difficult to sample, thus, sampling was restricted to the upper intertidal zone.

Portobello bay (-45.8385, 170.6492) is a small inlet located at the foot of the Portobello peninsular and opposite to Port Chalmers, a full-service port that has a history of intensive land reclamation and shipping channel modifications (Smith *et al.* 2010, Vial 2010). The sediment dominated seabed descends slightly as it extends into the harbour before dropping steeply ~300 meters offshore. An inlet flows down from the surrounding hills, acting as the only source of freshwater (Ismail 2010, Vial 2010). Seagrass was present in rough patches along the eastern side, concentrated towards the mid intertidal zone and extending along Gills Corner. At the foreshore, big portions of the beds were dead or dying and while dense patches were present, there were scarce throughout the area. It should also be noted that at this site, there was a persistent occurrence of oily residue and scum on the surface of the water.

3.2.3 Data collection

Community composition for seagrass and bare sediment habitat was assessed using a modified 1.2m x 1.2m seine net mounted on a PVC frame that can be hand-hauled. The net has a mesh of 0.05mm to avoid smaller organisms (eg juvenile fish spp.) being excluded. Precise coordinates and time at the

start and end of each tow were recorded on a GPS and were later transferred to computer using the application Basecamp by Garmin.

At the start and end of each tow, seagrass density was visually scored and categorized as either sparse, patchy, dense, or bare substrate. Sampling periods were chosen to allow maximum sampling time based on local tidal cycles and would commence just after high tide. While the beds were still submerged, sampling began at the intertidal zone and followed the outgoing tides in a series of non-overlapping tows pulled parallel to the shoreline. Tows were pulled throughout the intertidal zones, moving down with the retracting tide down to sample the subtidal zones.

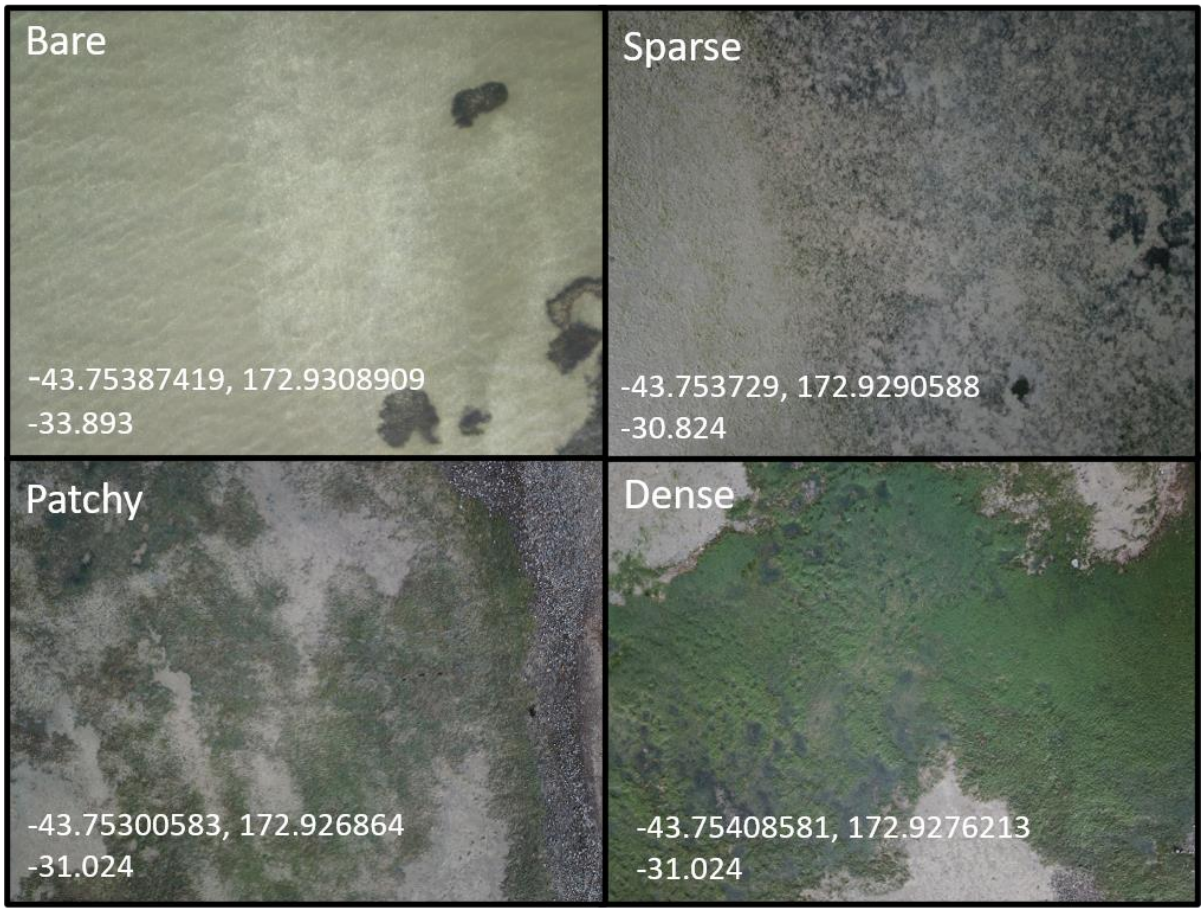

Figure 21: As seen in chapters 1 and 2, aerial drone shot representations of seagrass cover densities for sampling. Photos were taken at Duvauchelle bay during the autumn and include geographical coordinated in latitudinal/longitudinal form and geometric altitude (z)

Fig. 21. Aerial drone shot representations of seagrass cover densities for sampling. Also included are geographical coordinated in latitudinal/longitudinal form and altitude.

At the end of each tow, the net was sorted through and all living organisms were identified, counted, and recorded. To keep track of different developmental stages, several species were categorized into sizes of >10mm, 10-20mm, 20-50mm, and >50mm using a 0-50mm scale on the frame of the net. Species that could not be identified in the field were photographed for later identification. Percentage

of live seagrass roots in the net is visually estimated to give an indication whether seagrass is being uprooted by the net. From the experience of myself and my sampling team, we can conclude this method causes minimal to zero impact (Thomsen et al 2020).

3.2.4 Data analysis

To standardize for differences in sampling effort, species counts for each observation were divided by the area of each respective tow and expressed as species density or units per area. The area of each sample was calculated by taking the length of each tow (provided by Garmin GPS coordinates) and multiplying by the width of the net (1.2m).

A variety of approaches were taken to calculate diversity per site, region, cover density, and degree of disturbance. Initial diversity measures calculated on abundance data included species richness, Shannon-Weiner (Eq 1), Brillouin index (Eq 2), Peilou evenness (Eq 3), rarefied richness (Eq. 4) and effective (true) diversity. As sample randomness could not be guaranteed, the Brillouin index, a modified version of Shannon-Wiener, was measured to quantify entropy within this system where n_i represents the number of individuals and $\boldsymbol{N}$ representing the total number of individuals (Peet and Robert 1975).

Equation 1

$$H' = -\sum_{i=1}^{R} Pi \ln pi$$

Equation 2

$$H_B = \frac{\ln N! - \sum \ln ni!}{N}$$

Peilou evenness (J) is a popular measure of community homogeny and was used to compare the actual value of diversity (such as Shannon (H'), or in this case Brillouin (H_B)) to the possible maximum diversity value, where S is the total number of species (Jost, 2010, Pielou, 1966). Communities that have a high degree of evenness ted to have species that occur equally, though, it should be noted that J is highly sensitive to sample size and rare species (Jost 2010). All initial measures of diversity stated above were completed in Rstudio using the packages vegan (), and plyr ().

Equation 3

$$J = \frac{H_B}{H_{max}} = \frac{H'}{\ln S}$$

Given the differences between sample sizes, rarefaction was used to estimate, and plot expected number of species (*E(s)*) without the influence of sampling effort (Eq.4). This is a popular approach taken by ecologists as rare species are often overlooked if sampling effort is not high enough. Marine ecosystems in particular are considered sensitive to this issue as they typically have very high phyogenetic diversity, thus, a higher sample number is required (Foggo *et al.* 2003, Grassle *et al.* 1991). The rarefaction measure as proposed by Heck et al (1975), was used to generate random sub-samples (*n*) of nominated size (*N*) for a given taxa to give an approximate estimate of species richness.

Equation 4

$$E(s) = \sum 1 - \left[\frac{\frac{N - Ni}{n}}{\frac{N}{n}} \right]$$

Additionally, multiplicative group-wise diversity partitioning was calculated using the R package heirDiversity (Marion *et al.* 2015) and was used to quantify within-group (alpha), among-group (beta), and total diversity (gamma) in a hierarchical framework (fig.20) (Jost 2006). For each model 999 bootstraps were used to approximate uncertainty. This approach summarizes community complexity as Hill number which offers biologically relevant estimates of diversity and utilises hierarchical bootstrapping to approximate uncertainty for said estimates (Hill 1973, Marion et al 2015).

Direction and magnitude for relationships between environmental variables and community assemblages were examined using a distance-based redundancy ordination analysis (dbRDA) on Bray-curtis similarity matrices. dbRDA is a form of constrained ordination that resembles regular redundancy analysis (RDA) but differs in its ability to utilise several ecologically meaningful distance measures rather than solely Euclidean distances (Legendre and Anderson, 1999). As Euclidean distances can be an inappropriate representation for community response data, dbRDA has become increasingly popular among community ecologists as a more reliable approach for analysing species-environment relations. This provides a more accurate portrayal of community compositional data in a multivariate framework, particularly when working with species abundance datasets that are highly zero-inflated (Legendre and Anderson, 1999).

All ordination and PERMANOVA analysis was performed in Rstudio using the package vegan (). In this study, dbRDA was used to analyse variations between seagrass communities among regions, site, and seagrass cover. Species were further split into a separate dataset for fish and analysed separately to assess more specific patterns between fish distributions and abiotic factors. Subsequently,

PERMANOVA hypothesis testing was performed to assess the significance of our model and predictor variables. Triplots and biplots for each model were generated to visualize these patterns in multidimensional space, using species and site scores. NMDS ordination plots were also generated to graphically represent multivariate dissimilarity between regions.

For each dataset, spatial variations on abundance/density data were tested using a PERMANOVA permutational multivariate analysis of variance (PERMANOVA) approach using the Bray Curtis similarity matrix. PERMANOVA is a non-parametric statistical test that is best described as a geometric partitioning of multivariate variation where permutation is used to evaluate the significance of pseudo-F-ratio (Anderson 2017). In ecological data sets, assumptions of multivariate analysis of variance (MANOVA) are often not met. Convers Due to zero inflation and multicollinearity in the dataset, PERMANOVA was used to test whether community abundances differed based on latitude (Anderson 2017, Morrison *et al.* 2014).

Tests for main effects and interaction terms were run for each dataset. Subsequently, pair-wise tests were run to assess the significance of contribution for group differences. All PERMANOVA analysis was performed in Rstudio using the adonis () function from the Vegan package for community ecology analysis. Each model was generated using the Bray-Curtis method to calculate pairwise distances and partial R^2 (estimated variance explained) and P values were generated based on 999 permutations.

4.3. Results

4.3.1. Overall patterns for seagrass faunal communities

Across all regions, a total of 7214 individuals from 31 species were caught and identified. Of those 31, 12 species were shared across all regions and only a single species (mudflat top shell) was shared by all 9 sites. Southern sites as a collective had a species richness of 22, followed by Northern and Central sites which both had a collective richness of 18. Average faunal density was markedly higher in northern (11.97) and central (6.79) sites compared to southern sites (3.34). This inflation is likely the result of exceedingly large populations of shrimp which made up 38.8% and 66.5% of individuals caught for northern and central sites, respectively. In addition, northern sites were characterized by large numbers of arrowhead crab (18.5%), mud crab (7.6%), and southern creeper (15.9%), though density of each varied per site (table 4).

Table 5: Total and average density of species per site. Counts were first converted into a measure of individual density by dividing abundances by sampling area (m^2). Densities were then averaged by dividing by the number of samples. Sections correlate to different taxonomic classes (bony fish, crustaceans, gastropods, bivalves, and misc. invertebrates.

	North			Central				South	
	Thomp	Del	NelsH	Duv	Rob	AvonH	Waip	Porto	PapInlet

Sand flounder (+) (*)	8.77 [0.438]	-	1.42 [0.071]	2.46 [0.102]	1.74 [0.086]	0.68 [0.031]	1.55 [0.071]	0.48 [0.021]	4.05 [0.193]
Blenniiformes (+) (*)	1.05 [0.052]	-	-	1.07 [0.044]	0.73 [0.036]	-	3.41 [0.155]	0.74 [0.032]	-
Cling fish	-	0.15 [0.007]	-	-	-	-	-	-	-
Black goby	-	-	-	-	-	-	0.43 [0.019]	-	-
Blue cod (*)	0.16 [0.007]	-	-	-	-	-	-	-	-
Banded wrasse (*)	-	-	-	0.22 [0.030]	0.26 [0.013]	-	-	-	-
Spotty (+) (*)	0.21 [0.011]	-	-	0.73 [0.009]	-	-	0.43 [0.019]	-	-
Wide-bodied pipefish (*)	-	-	-	0.08 [0.003]	-	-	-	-	-
High-body pipefish	-	-	-	0.05 [0.002]	-	-	0.06 [0.003]	-	-
Long-snouted pipefish	-	-	-	-	-	-	0.06 [0.003]	-	-
Shrimp *spp.* (+)	28.78 [1.439]	48.31 [2.543]	0.37 [0.018]	53.51 [2.23]	40.54 [2.027]	-	0.10 [0.004]	-	-
Pillbox crab (+)	26.18 [1.309]	9.02 [0.475]	8.42 [0.421]	2.02 [0.091]	1.82 [0.009]	0.68 [0.031]	1.78 [0.081]	0.10 [0.004]	-
Burrowing mud crab	17.56 [0.878]	0.95 [0.050]	0.30 [0.015]	0.34 [0.023]	0.46 [0.023]	1.99 [0.091]	-	-	-
Stalk-eyed mud crab (+)	7.86 [0.393]	0.13 [0.007]	0.47 [0.023]	0.21 [0.021]	0.43 [0.021]	1.75 [0.079]	2.54 [0.115]	-	0.10 [0.005]
Hermit crab	3.31 [0.165]	-	-	-	-	-	-	-	-
Isopod *spp.*	-	3.30 [0.174]	0.66 [0.033]	-	-	-	-	-	-
Sea centipede (+)	-	1.88 [0.021]	-	0.428 [0.019]	-	-	10.73 [0.468]	0.20 [0.54]	-
Mudflat top shell (+)	3.41 [0.171]	1.88 [0.099]	0.11 [0.005]	2.042 [0.085]	1.726 [0.086]	4.07 [0.185]	9.04 [0.411]	4.64 [0.202]	[0.265]
Mud snail	-	-	-	0.455 [0.056]	1.110 [0.056]	2.83 [0.128]	0.05 [0.002]	-	0.10 [0.005]
Cats eye	-	-	-	-	-	-	-	2.00 [0.087]	-
Bubble snail	-	-	-	-	-	-	0.18 [0.008]	-	-

Mud whelk	1.52 [0.076]	0.03 [0.002]	1.83 [0.092]	0.855 [0.036]	0.895 [0.045]	-	-	3.96 [0.172]	1.76 [0.084]
Turret shell	-	-	-	-	-	-	2.66 [0.122]	-	-
Southern creeper (+)	8.98 [0.449]	-	37.46 [1.873]	0.435 [0.018]	-	4.39 [0.20]	0.09 [0.004]	0.14 [0.006]	0.12 [0.006]
Cockle (+)	5.23 [0.262]	2.14 [0.113]	1.3 [0.056]	2.825 [0.118]	1.804 [0.090]	0.90 [0.041]	0.64 [0.29]	15.56 [0.676]	0.13 [0.006]
Pacific oyster	-	-	-	-	-	-	-	-	0.22 [0.011]
Pipi	2.70 [0.135]	-	-	-	-	-	0.05 [0.002]	-	-
Cushion star	1.23 [0.061]	-	-	-	-	-	-	-	-
Sea squirt	-	-	-	0.428 [0.018]	-	-	-	-	-
Bristleworm	-	-	-	0.152 [0.006]	-	-	0.04 [0.468]	-	-

(+) indicate species that are shared across regions, (*) represents juvenile species present, - represent 0

Table 6: Total and average [] density of taxonomic classes per site (See table 1 for species groupings). Raw counts were converted into a measure of individual density where abundances were divided by sampling/tow area (m^2). Densities were further divided by the number of samples to calculate average density.

	North			Central			South		
	Thomp	Del	NelsonH	Duv	Rob	AvonH	Waip	Porto	PapInlet
Bony fish	4.70 (0.21)	0.10 (0.01)	1.51 (0.08)	5.31 (0.22)	2.96 (0.15)	1.52 (0.07)	4.70 (0.21)	1.61 (0.07)	4.57 (0.22)
Crustacean	3.80 (0.17)	59.55 (3.13)	10.08 (0.50)	68.18 (2.84)	38.00 (1.90)	4.20 (0.19)	3.80 (0.17)	0.11 (0.005)	0.10 (0.004)
Gastropod	11.50 (0.52)	2.20 (0.12)	26.27 (1.31)	3.63 (0.5)	4.19 (0.21)	7.27 (0.33)	11.50 (0.52)	10.82 (0.47)	9.41 (0.45)
Bivalves	1.47 (0.07)	1.40 (0.07)	6.17 (0.31)	5.00 (0.21)	1.33 (0.07)	1.19 (0.05)	5.85 (0.27)	4.67 (0.20)	0.89 (0.04)
Misc. invertebrates	0.51 (0.03)	0.33 (0.02)	- (-)	1.49 (0.06)	0.32 (0.02)	- (-)	8.89 (0.40)	1.16 (0.05)	- (-)

For seagrass communities as a whole, 39.3% of variation is explained by distance-based redundancy analysis, leaving 60.7% unconstrained. Overall, 15.56% and 6.51% of variation was explained by the first and second axes accordingly (fig. 4). PERMANOVA hypothesis testing revealed that significant relationships between species composition and all of the explanatory variables (P=0.001) (table 9). PERMANOVA testing of canonical axes show that seagrass communities as a whole are highly significant along the first four axes (P(>F) =0.001), nevertheless, most variation seen remains in the first two axes. Thus, plotting by the first and second axes in multidimensional space should represent the model best. Further hypothesis testing (table 5) show that all predictor variables for both models displayed significant p-values (P(>F) =0.001***), however, site appears to have greater dispersion (F=10.448) compared to region and cover (F=2.261).

Table 7: PERMANOVA hypothesis testing of predictor variables for each dbRDA community model. In PERMANOVA, random permutations of objects in the species matric to generate a null distribution (H0) which detects linear relationships between predictor and response variables. Different aspects of the model can be tested for significance using this method.

		df	SS	*pseudoF*	Pr(>F)
Seagrass					
	Region	2	1.786	1.8874	0.001***
	Site	6	17.117	10.448	0.001 ***
	Cover	3	1.852	2.261	0.001 **
	Residual	179	48.876		
Fish					
	Region	2	2.2601	3.1479	0.001***
	Site	6	5.9186	4.5560	0.001***
	Cover	2	2.3273	3.5829	0.001***
	Residual	104	21.4351		

Abbreviations: SS, sum of squares; MS, mean sum of squares

Significance based on 999 permutations.

Significance level: <0.1*, <0.01**, <0.001***

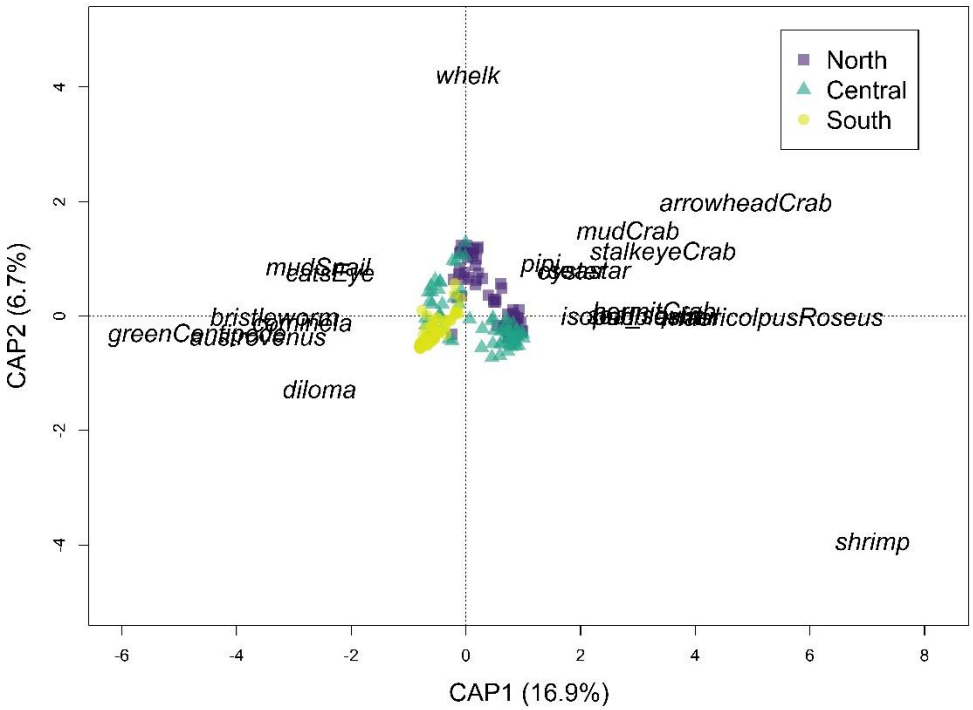

Figure 22: Distance-based redundancy analysis plots for entire communities across regions, cover types and site. Species scores were plotted against site scores to detect any regional patterns in community composition. The magnitude for relationships between variables and species is illustrated based on the length and the direction of the scores from the centre.

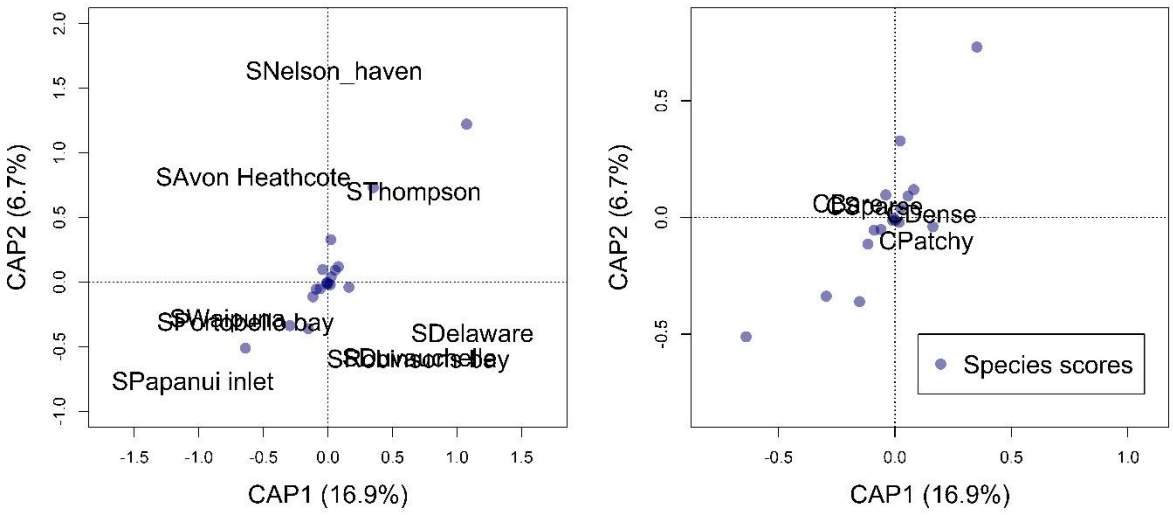

Figure 23: Distance-based redundancy analysis biplots of species scores across site and seagrass cover types. Plots were split into two for clearer visualization of the magnitude that environmental variables have one species distribution.

As shown in Figures 5, several clusters have arisen for regional scores where north and south appear to cluster within, whereas, central shows more shared variation

Scores for blenniformus, commonly known as blennies cat's eye *(Lunella smaragdus*), turret shell (*Maoricolpus roseus*), mud whelk (*Cominella glandiformis*), bubble snail (*Bulla quoyii*), mudflat top shell (*Diloma subrostratum*), and green marine isopod (*Paridotea ungulata*) have clustered near and within southern site scores (fig. 22). Additionally, cockles (*Austrovenus stutchburyi*), southern creeper (*Zeacumantus subcarinatus*), and shrimp are spaced away from the centre and species clusters, suggesting these species display larger differences from other species. There is a slight overlap between central and southern replicates, suggesting that these regions share similarities in species composition. On the other hand, northern replicates were characterized by crab species, as shown in figure 22. In multidimensional space, site appears to play a bigger role in determining species composition than cover type (fig. 23 a and b). In figure 5b, scores for cover type split into clusters of patchy-dense and bare-sparse cover though the difference between clusters is small.

When treated as its own community, the fish model explained 20% of variation seen.
For fish communities, 17.48% of variation in the redundancy-based model was explained by the first axes, with 6.45% explained by the second. Juvenile sand flounder (*Rhombosolea plebeia*) and blennies are positioned far from other species clusters, indicating they have a greater influence on the model than other species caught (figure 7a). These deviations are due to the high catch rate of flounder and blenniformus for all regions. Upon closer examination (fig. 7b), species such as blue cod (*Parapercis colias*), spotty (*Notolabrus celidotus*), and wide-bodied pipefish (*stigmatopora nigra*) have clustered closely together, with juvenile wrasse (*Pseudolabrus fucicola*) and high-bodied pipefish (*Leptonotus elevatus*) positioned nearby. Clingfish, black goby, blue cod, and long-snouted pipefish also cluster together, but no obvious regional patterns can be seen.

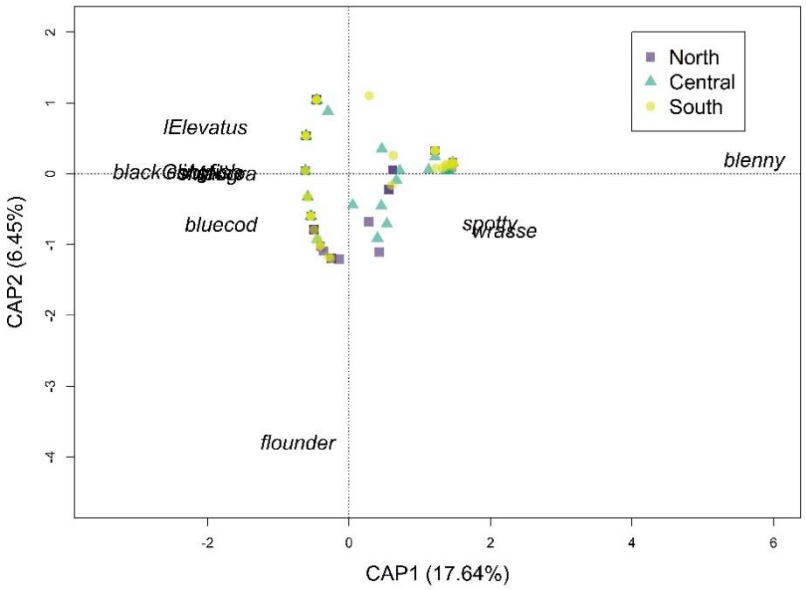

Figure 24: Distance-based redundancy analysis plots for fish communities analysed across regions, cover types and site. Species scores were plotted against site scores to detect regional patterns in community composition. The magnitude for relationships between variables and species is illustrated based on the length and the direction of the scores from the centre.

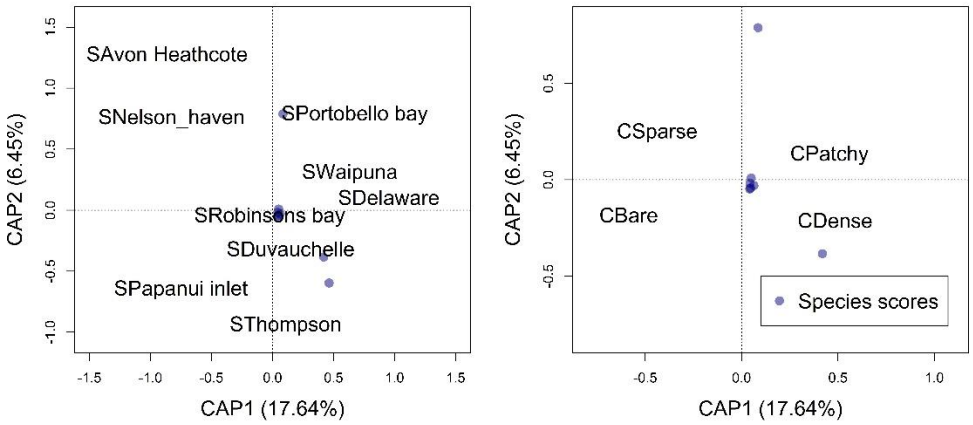

Figure 25: Distance-based redundancy analysis plots for fish communities across sites and seagrass cover types. Plots were split into two for clear visualization of patterns between fish species and environmental variables (8a and b). The magnitude for relationships between variables and species is illustrated based on the length and the direction of the scores from the centre.

Ordination plots for species scores against site and cover show that models for seagrass and fish differ considerably. For example, southern and central site scores for seagrass communities cluster closely together, whereas site scores for fish communities are spread. This may signify that fish communities vary more over sites that seagrass communities as a whole. Similarly, seagrass cover types have an overall greater influence on fish than seagrass communities (fig. 25). This suggests that each cover type has differences in fish composition. Based on positions, bare and patchy habitats are highly dissimilar, whereas bare and dense cover type is less so. This model does, however, display a

horseshoe effect. Horseshoe effects often arise in biological datasets if species have a unimodal response curve, and if beta diversity is moderate to high (Bocard *et al.* 2011). Therefore, this model may not be appropriate for determining multidimensional patterns in seagrass associated fish populations across regions.

4.3.2) Latitudinal patterns of diversity

PERMANOVA results (table 4) show that region has considerable influence on community structure for both models. Due to having higher statistical power, effects were distinctly higher for seagrass communities as a whole (PERMANOVA, p=0.001***). The majority of variation for each model is explained by the residuals, which contributed 46.7%and 53.4% for seagrass and fish models, accordingly. Partial R^2 values further show that region explained 19.1% of variation for seagrass communities which is notably higher than other variable effects. In contrast, out of all the significant effects, region contributed the least amount of variation for fish assemblages.

*Table 8: Main and interaction effects for communities using a fixed PERMANOVA design. PERMANOVA is a distance-based method that partitions diversity from a distance matrix among sources or variation. Predictor variables include region (fixed with three levels) and seagrass cover (fixed with four levels). The pseudo-f statistic is calculated by dividing the explained variation by the unexplained variation; hence, larger F values indicate more pronounced separation between groups. P-values represent significance, which is annotated by the number of *, whereas R2 values represent the proportion of variation in the model explained by predictor variables.*

	Seagrass				**Fish**			
	df	Psuedo - F	R^2	p-value	df	Psuedo - F	R^2	p-value
Region	2	21.7202	0.18547	0.001***	2	3.1378	0.05056	0.005**
Cover	3	1.9088	0.02445	0.006***	3	3.7550	0.09075	0.001***
Region x Cover	6	1.0080	0.02582	0.459	6	1.2652	0.06115	0.180
Residuals	179		0.76426		77		0.79754	
Total	190		1.000		110		1.000	

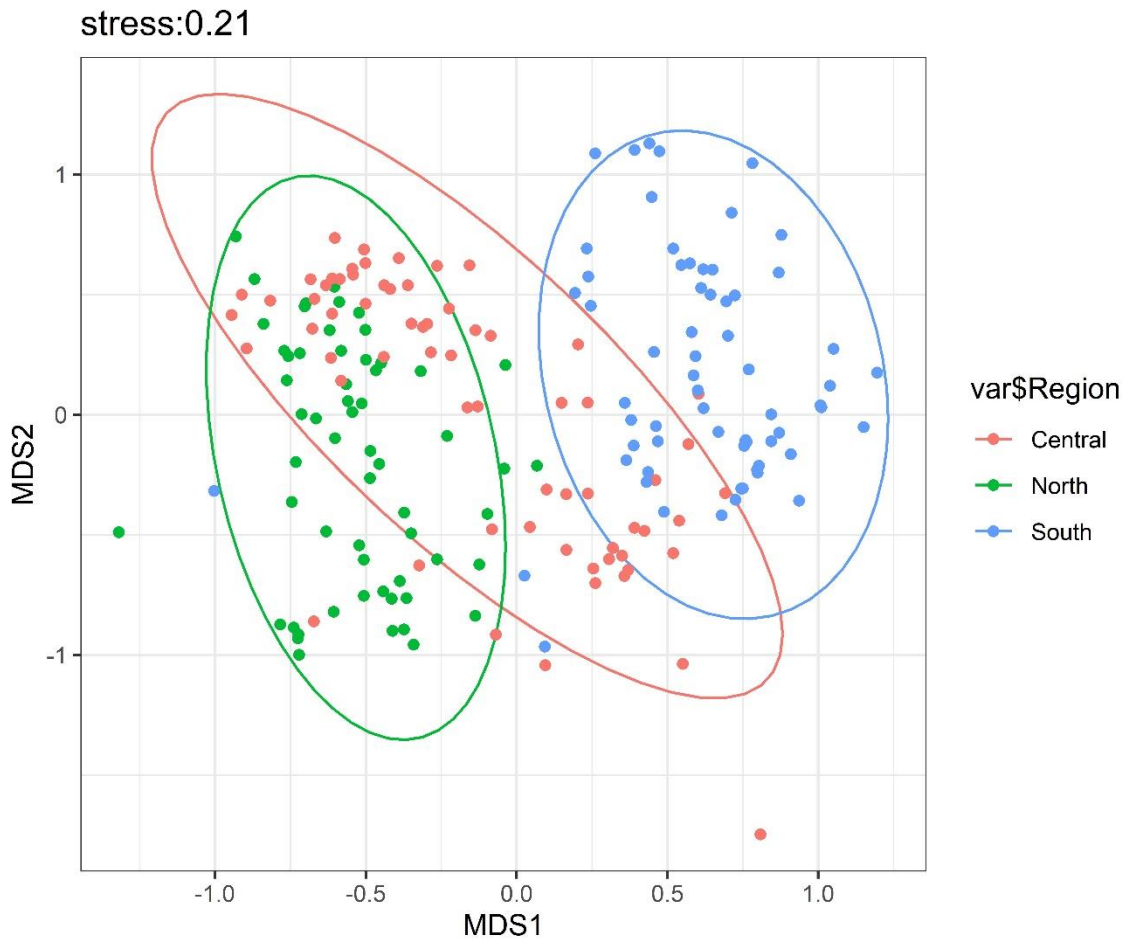

Figure 26: Non-metric multidimensional scaling (NMDS) plot illustrating differences in species composition among samples and between regions (North, Central, South). Ellipsoids illustrate the standard deviation of weighted averages for each region based on their associated NMDS scores. Prior to plotting, scree plots were generated to determine the optimal number of dimensions required to best visualise patterns in the data. The stress value (0.14) is an approximate representation for the model's "goodness of fit" and should ideally be less than 0.2 for the model to be considered of good fit.

Overall, 12 species were shared across all three regions, though faunal density varied widely across sites and species. Species such as flounder, cockle, mudflat top shell, pillbox crab and stalkeyed crab were present and highly abundant across all regions, whereas, other species were only occurred in one or two regions at lowered abundances (table 4.). Regional variation in species composition showed that northern and southern sites were highly dissimilar, whereas central shared variation with both other regions as would be expected when assuming latitudinal patterns (fig. 26). North and central shared a considerable amount more of variation than that of central and south, though these similarities were likely driven more by faunal abundances than by the presence/absence of species (fig. 26). For example, northern and central sites exclusively share 1 species (burrowing mud-crab), whereas southern and central share 3 (bristle worm, high-bodied pipefish, mud snail). Be that as it may, species such as shrimp, pillbox crab, stalk-eyed mud-crab, southern creeper, and cockles displayed remarkably high faunal densities in northern and central communities (table 4).

Table 9: Mean density, effective richness, and diversity measures for each site. Effective diversity was calculated as a function of q, where q=1 represents Shannon diversity and q=2 represents Simpsons diversity. As sample randomness could not be guaranteed during sampling, Brillouin diversity was also calculated for each site. Brillouin diversity is a modified version of the Shannon-Weiner index that is often preferred when sample randomness cannot be achieved. Furthermore, Peilous evenness provides an indication as to whether species are all equally common by comparing the actual diversity value to the possible maximum diversity value.

Region	Site	Mean density	Effective richness (q=0)	Diversity order (q=1)	Diversity order (q=2)	Brillouin index (H_B)	Peilous evenness (H')
North	Thompson	116.95	15	8.451	1.837	2.065	0.772
	Delaware	66.31	10	2.676	1.805	0.902	0.399
	Nelson Haven	52.16	10	2.734	1.837	1.041	0.466
Central	Duvauchelle	68.86	18	2.908	1.641	1.007	0.357
	Robinson	40.29	12	2.749	1.623	0.974	0.405
	Avonheathcote	8.10	6	6.028	5.680	1.777	0.897
South	Waipuna	34.39	19	7.177	5.161	1.933	0.674
	Portobello	29.06	10	4.299	2.956	1.436	0.640
	Papaunui inlet	12.04	8	3.491	2.868	1.199	0.604

Standardised values of beta diversity (turnover) confirm this. At q=0, species turnover rates are notably higher for grouped southern sites than that of north and central (fig. 29) As q increases and rare species are down weighted, this divergence is reduced, resulting in all regions sharing similar rates of turnover when q≥1.Overall, despite having a higher species richness, the south had heavily reduced faunal density when compared to other regions (table 4). Furthermore, as shown in figure 27, southern sites are clustered together exclusively while central and northern communities are intermingled among each other. When comparing assemblage across sites we see that the Avon Heathcote and Nelson-haven estuary (both areas of high disturbance) have clustered together exclusively rather than with sites within their respective regions. If we cluster the data via region (fig. 27), central and north group together within the same branch further indicating the similarity between these regions, however, as the unbiased p-value (89%) is less than 95%, this relationship is not significant at an alpha of 0.05.

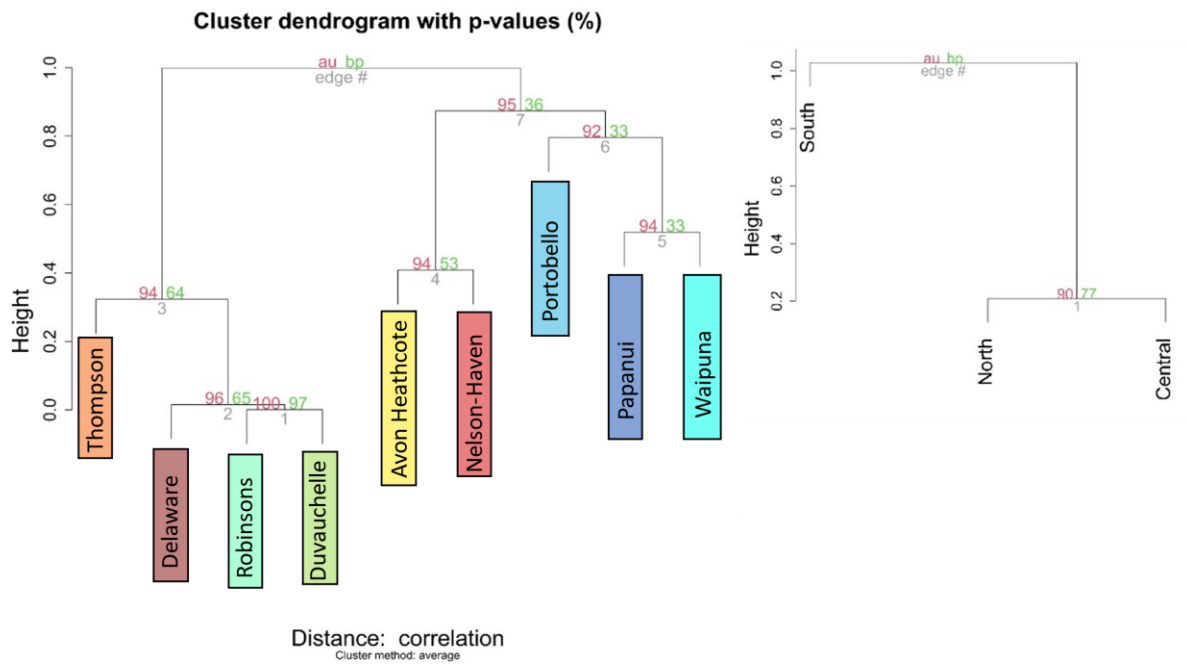

Figure 27: Dendrogram of ecological differences for faunal seagrass communities across nine sites using the Bray-curtis dissimilarity matrix. Au values in red represent the approximate unbiased probability values computed through multiscale bootstrap resampling), while bp values in green represent bootstrap probability estimates. Sites are coloured accordingly to their corresponding region.

In contrast to the hypothesis, lateral diversity and richness were highest in the south and lowest in the north (fig.28 and 29a). Despite having a relatively low mean density of individuals caught, Waipuna bay in the south displayed the highest interpolated and extrapolated estimates for species diversity and richness across all sites, followed closely by Duvauchelle bay and then Thompson bay (table 4). Rarefied estimates show that sites across the northern and central regions reached a maximum for true diversity (q=0) within the number of individuals caught during sampling. As seen in figures 28a and b, low disturbance sites for these regions start reaching the maximum diversity at approximately 1500 individuals caught. Contrarily, estimates for Waipuna bay reached a maximum extrapolated species diversity of 27 at approximately 3000 individuals caught (fig. 28c). Thus, higher sampling effort is recommended for accurate representations of diversity in this site. Moderate and high disturbance sites across all regions seem to be well within the estimated number of individuals caught to achieve maximum diversity (fig. 28a, b, and c). Though the south has higher effective richness, alpha diversity values indicate that average species diversity is lowest in the south and higher in the north and central regions when q=0 (fig. 29). As rare species become down-weighted, differences in alpha between regions become less pronounced until they are indistinguishable when q<1.

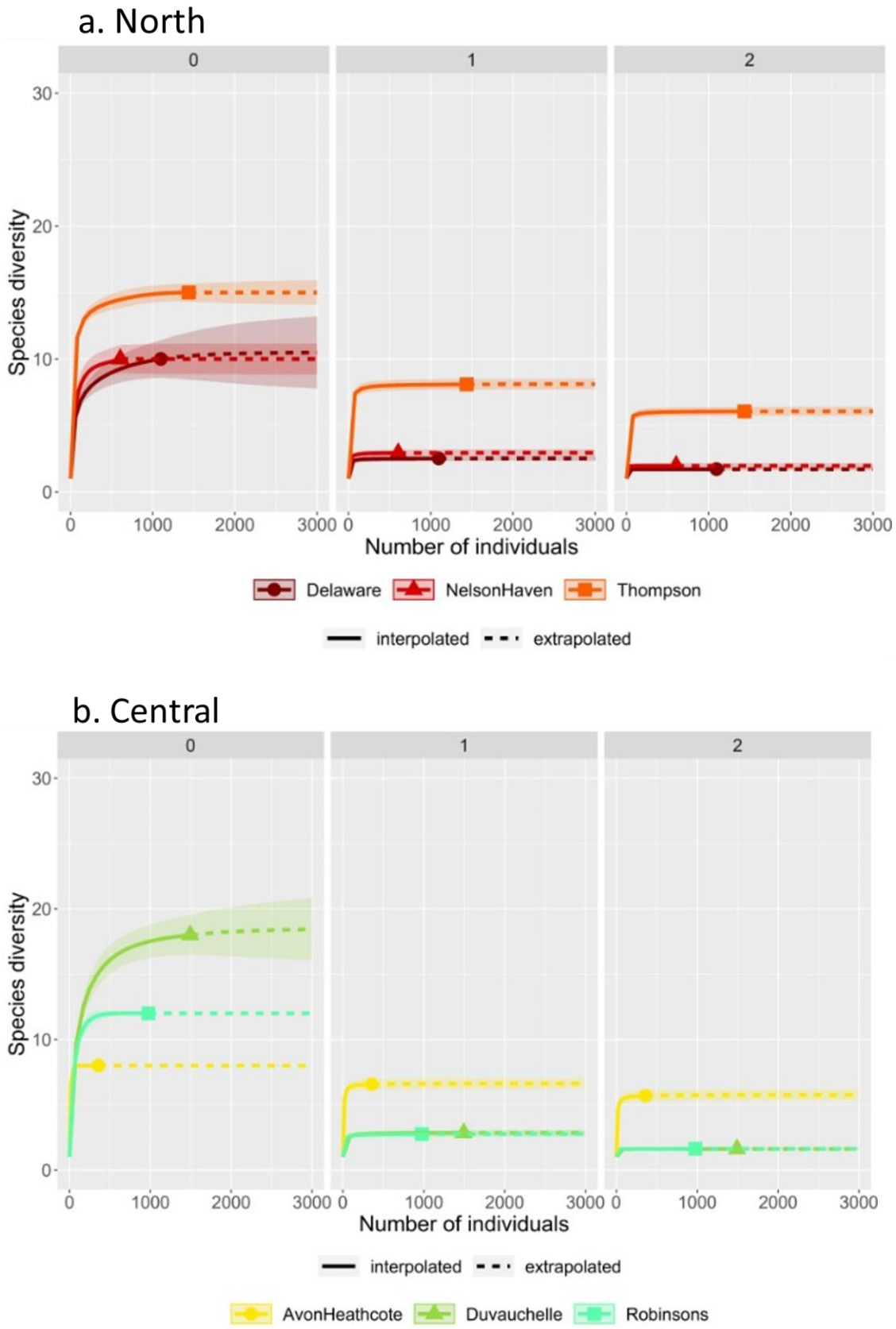

Figure 28: Rarefaction curves for northern (a), central (b) and southern (c) sites. Assigned levels of disturbance of each site are indicated by colour. Solid lines represent the actual data while dotted lines represent the estimated values of species diversity without the limitation of sample size. Curves for each site were also generated as a function of Shannon (q=1) and Simpsons (q=2) diversity.

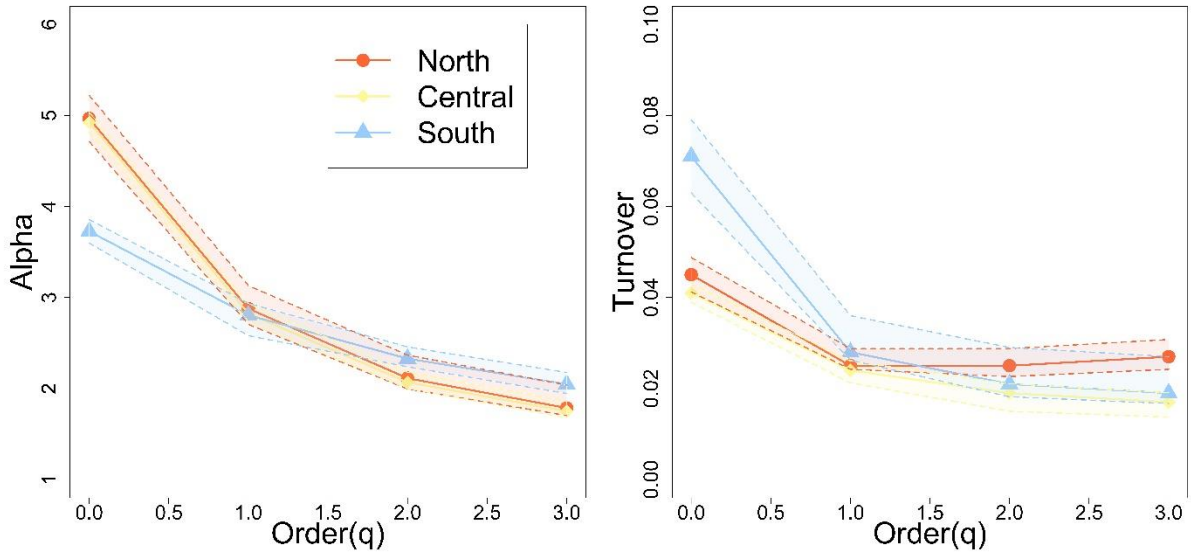

Figure 29: Hierarchical, group-wise regional diversity profiles where estimates for alpha (a) and turnover (b) are plotted as a function of order q. Parameters for q define the down weighting of rare vs common species. As the value of q increases, the effective weight is assigned to the most common and abundant species. Thus, as q increases, the influence of rare species is suppressed. Values of q also represent orders of diversity where q=0 corresponds to the estimates of true diversity (weighted harmonic mean), q=1 to the Shannon index (weighted geometric mean) and q=2 to the Simpson index (weighted arithmetic mean).

4.3.3) Variation across sites

PERMANOVA analysis (table 4) detected strong main effects for region and cover (p=0.001***) across both models, with no significant interaction detected between region and cover. Partial ^{R2} values show that independently, region contributes 18.54%, to variation for seagrass communities, while cover and the interaction between cover and region explained.

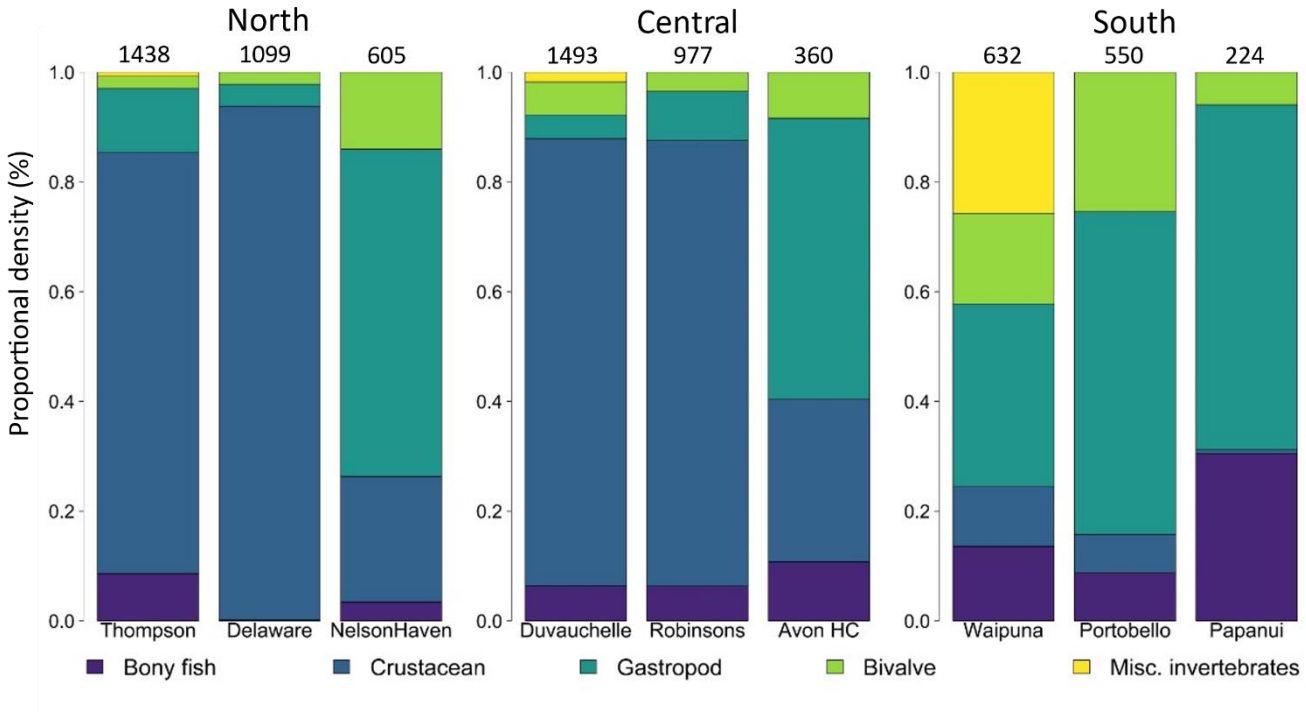

Figure 30: Proportional density of classes across all regions. Each graph represents the different regions (north, central, south) where sampling occurred. Species were grouped based on taxonomic class and converted into a measure of density by dividing raw counts by the sampling area (m2) before being calculated into proportions. Names for sites can be located in table 2.

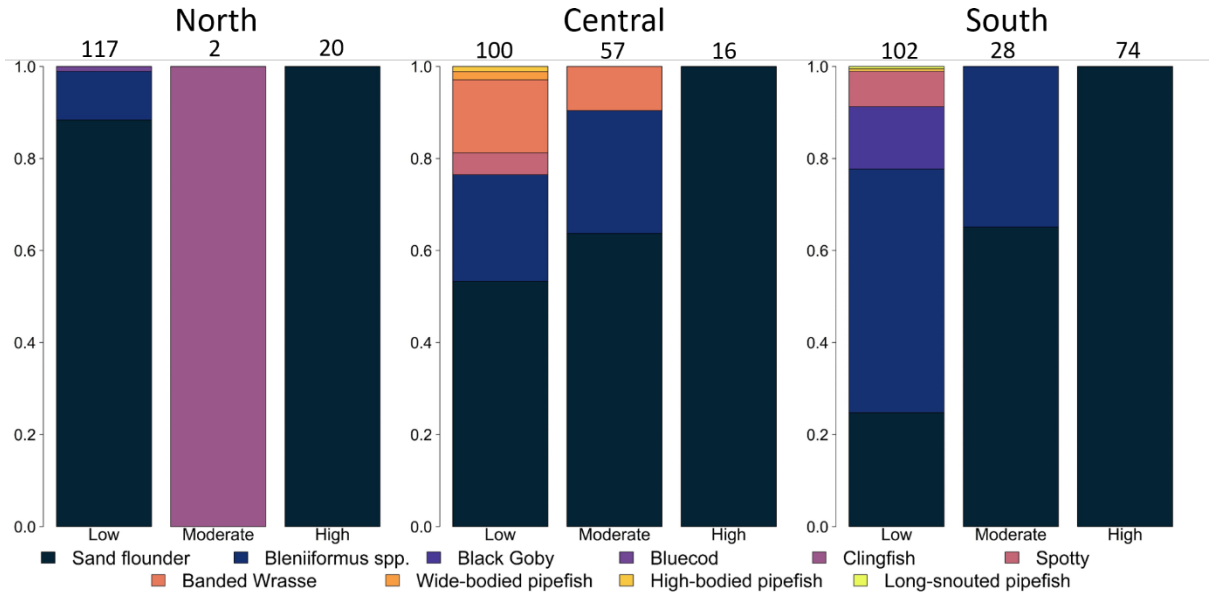

Figure 31: Proportional density of fish species for low, moderate, and high disturbance across all regions. Raw counts were converted into a measure of individual density by dividing counts by sample area before being calculated a proportions.

Across regions, taxonomic groups for low sites were characterized by bony fish, crustaceans, gastropods, bivalves, and miscellaneous invertebrates. Thompson bay held the highest mean density of individuals caught, which is approximately double what was caught in Delaware Bay and Nelson

Haven (table 3). Within each region, one site exhibited considerably higher mean densities of individuals caught compared to other sites sampled, though this was not the case for the south. For example, despite Waipuna Bay having the highest species richness, the average density of individuals caught did not deviate significantly from Portobello Bay or Papanui inlet (table 4).

In addition, isolated sites supported the richest fish communities out of all sites sampled, with 6 caught in Duvauchelle bay and Waipuna bay, 4 in Thompson bay and 9 caught in total (table 1 and 2). When comparing fish communities across all sites, most species caught occurred exclusively within low disturbance sites. This included three species of native pipefish, wide-bodied pipefish (*stigmatophora nigra*), long-snouted pipefish (*stigmatopora macropterygia*), and high-bodied pipefish (*leptonotus elevatus*) which were caught in Duvauchelle and Waipuna bay. For juvenile species, sand flounder made up the majority of the proportional density for northern (88.4%) and central (53.3%) sites, followed by species of blenny. which contributed 10.5% and 23.5% consecutively (fig. 14). Juvenile fish populations in Waipuna bay follow the opposite of this trend, where blenny were most abundant, followed by flounder (fig. 14).

While sites such as Robinsons bay and Delaware do not exhibit significant taxonomic shifts compared to sites in the regions, they did display lowered richness and mean density (table 3). As for the south, proportional density is comprised mostly of gastropod and bivalve taxa which were driven by the higher proportions of mudflat top shells mud whelks, and cockles caught in this area (fig. 13, table 2). Fish populations in Delaware bay consisted only of clingfish (*Trachelochismus melobesia*), a potential consequence of estuary hydrology (fast-flowing channels). On the contrary, Robinsons and Portobello bay were dominated by juvenile sand flounder, in addition to species of juvenile blenny and wrasse, though wrasse was only caught in Robinsons bay (table 2).

Beds in Nelson Haven, the Avon Heathcote, and Papanui inlet were found to have significantly reduced richness and overall abundances when compared to areas of low and moderate disturbance. Regions shared a similar shift in taxonomic dominance within seagrass beds from crustacean to gastropod species as disturbance increases. High disturbance sites across all regions were heavily dominated by gastropod taxa, which made up 59.7%, 51.2%, and 62.9% of individuals caught in Northern, Central, and southern sites respectively (fig. 13). It should be noted that overall, southern sites display higher densities of gastropod species, however, proportional density does increase considerably across treatments of disturbance (low – 33.2%, moderate - 58.7%, high – 62.8%). Though gastropods were the most prevalent taxonomic group in these areas, abundances consisted only of 2 or 3 species those being mud snails (*potamopyrgus antipodarum*), mud whelk (*cominella glandiformis*), and southern creeper (*zeacumantus subcarinatus*) (table 1). In comparison, these sites also displayed significant reductions in effective richness and mean density of individuals caught.

Furthermore, fish populations across all regions caught consisted only of juvenile sand flounder (fig. 14).

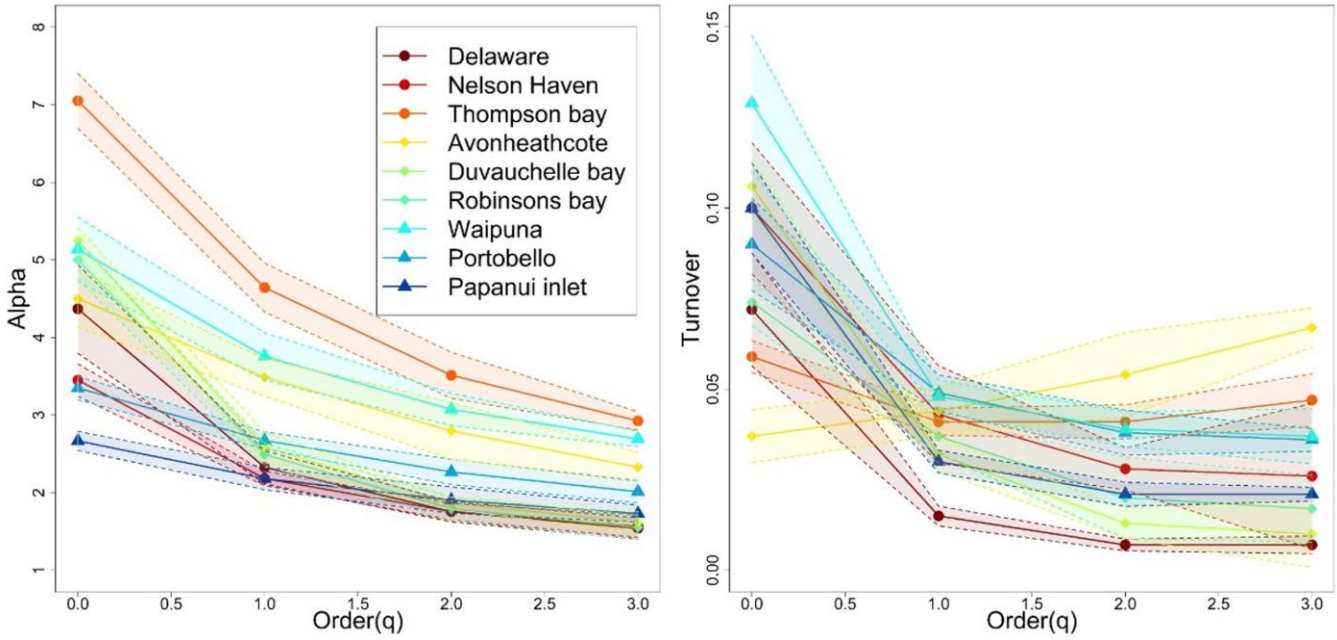

Figure 32: Hierarchical, group-wise diversity profiles for sites, where estimates for alpha (a) and turnover (b) are plotted as a function of order q. Parameters for q define the down weighting of rare vs common species. As the value of q increases, the effective weight is assigned to the most common and abundant species. Thus, as q increases, the influence of rare species is suppressed. Values of q also represent orders of diversity where q=0 corresponds to the estimates of true diversity (weighted harmonic mean), q=1 to the Shannon index (weighted geometric mean) and q=2 to the Simpson index (weighted arithmetic mean).

Profile plots show that alpha diversity when q=0 is markedly higher for Papanui, Portobello, and Nelson haven, whereas and high sites exhibit very similar values (fig. 15a). Despite numerous sites having a marginally higher alpha diversity than each other, the overlapping 95% confidence intervals suggest that such differences significant. Hierarchical partitioning of diversity found no distinct patterns for species turnover across diversity orders (q) between treatments. Without regard to the 95% confidence intervals, southern sites have marginally higher species turnover at q=0 others. As q increases, so does the turnover for the Avon Heathcote sites, whereas turnover for other sites decreased slightly after q=1 (fig. 15b). Nevertheless, these variations are not significant when accounting for the 95% confidence intervals.

4.3.4) Effect of seagrass cover

Overall, seagrass habitat supported more diverse and abundant faunal communities compared to unvegetated habitat. Species abundance for northern and central cites follow very similar patterns where dense patches supported the highest abundances, followed by patchy, sparse and bare respectively. Bare and sparse habitat do not appear to differ notably in abundance, however the

number of individuals caught doubled from sparse to patchy habitat (fig. 33). In contrast, southern sites more individuals were caught in patchy seagrass compared to dense. Both bare and sparse replicates also did not differ notably.

Significant differences in community diversity and abundance were detected between seagrass and bare sediment habitats with dense patches harbouring the highest average density of individuals. PERMANOVA partitioning (table 6) shows that treatments of seagrass cover had significant spatial influence on community assemblage across all 3 community models (p = 0.001***). Partial R^2 values for the main effects of cover explained 2.1% of variation for seagrass and contributed the least amount of

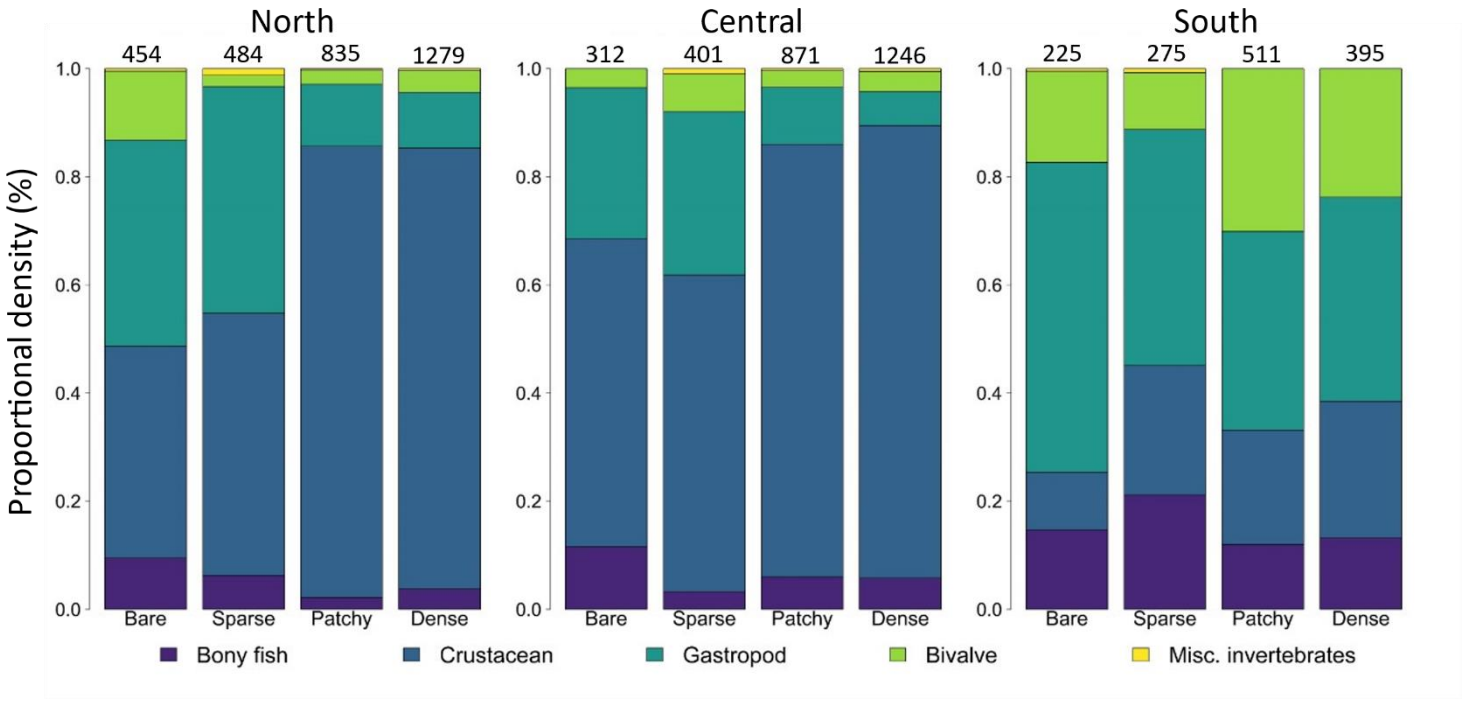

Figure 33: Proportional density of taxonomic classes across region and seagrass cover types (bare, sparse, patchy, and dense). Species were grouped based on taxonomic class and raw counts were converted into density counts before being calculated as proportions

variation for significant effects. On the contrary, cover contributed 7.1% to variation seen in the fish communities, which is more than what was explained by region. Though, it is worth noting that the difference between contributions put forth by cover and region is minor. Furthermore, the low proportion of variation explained for fish by cover is likely driven by the large numbers of flounder that were caught across all conditions. Similarly, while the impact for seagrass models is significant, it contributes much less to variation than that of other variables. PERMANOVA did not detect any notable interaction effects involving cover and region for both fish and seagrass communities and fish models accordingly.

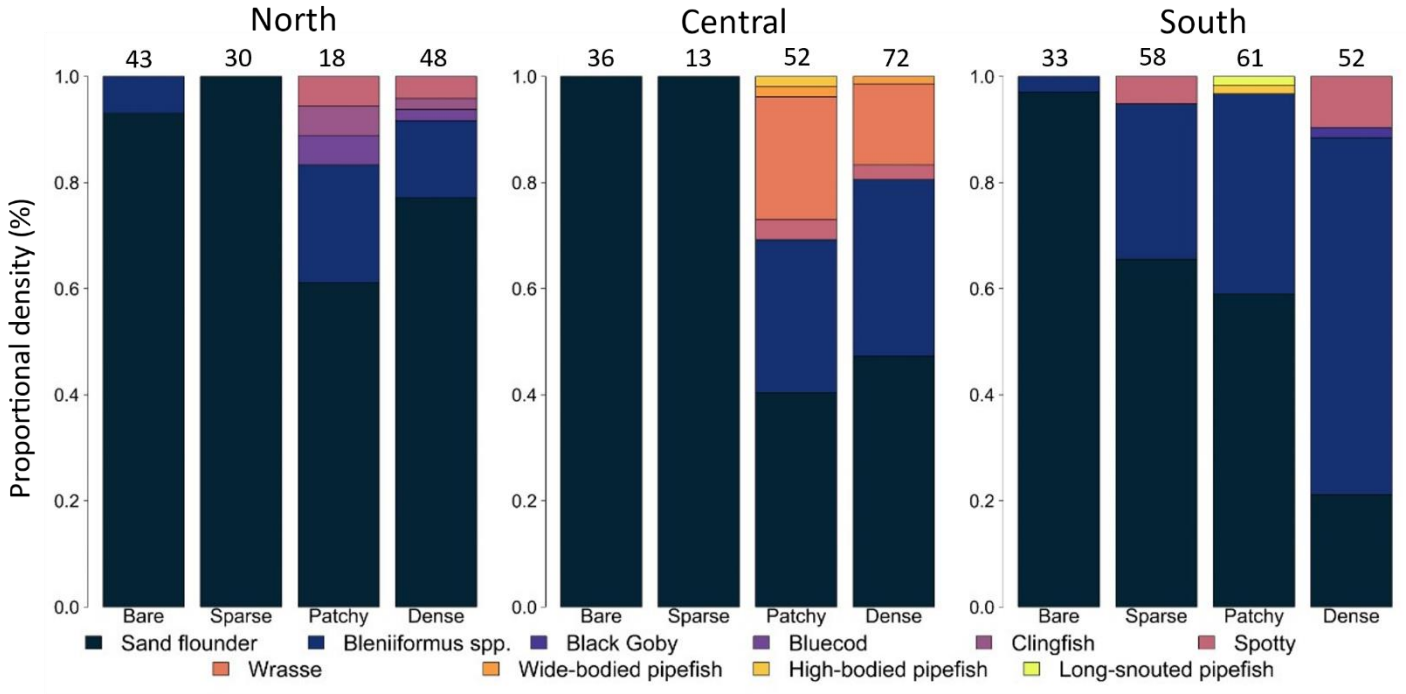

Figure 34: Proportional density (m^2) of classes for cover types (bare, sparse, patchy, and dense) across regions. Species were grouped based on taxonomic class and raw counts were converted into density counts before being calculated as proportions

Figures 17: Proportional density of fish species for cover types (bare, sparse, patchy, and dense) across regions. Species were grouped based on taxonomic class and raw counts were converted into density counts before being calculated as proportions.

Average density for all cover types in sites within close range of urban development (Nelson Haven. Avon Heathcote) was less significant than that of isolated sites, therefore, proportional density is subject to change with minimal changes in individuals caught (fig. 33). Such sites were dominated by distinctly high proportions of gastropod species across all levels of cover type, through patchy and dense replicates had increased proportional density of bivalves compared to sparse and bare sediment. With regards to the slight drop in proportional density as cover type increases, no distinguishable patterns were present for fish and crustacean communities (fig. 33). As stated previously, fish communities consisted mostly of sand flounder, which occurred in higher numbers within bare sediment replicates (fig. 34).

In contrast, sites such as Thompson bay, Duvauchelle bay, and Waipuna bay display a completely different taxonomic structure across habitat types, comprised mostly of crustaceans that increase in density in response to cover (fig. 33). For Thompson and Duvauchelle bay, this is heavily driven by disproportionately large populations of shrimp and crab species. While there are few detectable taxonomic differences between the two, only low disturbance replicates included cushion stars

(*patiriella regularis*), sea squirts (*pyura pachydermatina*), and bristle worms (*perinereis amblyodonta*) (table 1).

For sites that harboured substantial fish communities, fish were highest in richness and abundance in patchy seagrass cover, whereas only juvenile flounder and blenny were caught in bare sediment replicates. In bare sediment and sparse seagrass cover, juvenile flounder made up most of the fish caught (95.75%, 65.48%) (fig.34). Patchy cover has significantly reduced populations of flounder and is dominated mostly by blenny. The proportional density of flounder does; however, increase from patchy to dense cover by 13.37%. Species including all three pipefish were found exclusively in patchy, low disturbance habitat; however, this may be due to very few of these species caught during sampling. Dense patches supported populations of juvenile blue cod, spotty, and banded wrasse.

Overall, patchy seagrass cover supported the highest effective richness of species and average diversity. As shown in figure 35a, alpha diversity is highest in patchy replicates, followed by dense, sparse, and bare sediment habitat, respectively. Estimates for all cover types are highest at q=0 and decrease notable when q=1. Decreases continue steadily as q increases and rare species become more down weighted. This suggests that overall, communities are comprised of many rare species and few adundant species (fig.35a). At q=0, dense and patchy replicates are contributing more to beta diversity than bare and sparse replicates (fig.35b). As q increases, turnover for each cover type becomes more similar. At q=2, bare sediment suddenly rises and continues to increase; however, deviations in alpha and beta diversity cannot be considered significant due to the overlapping confidence intervals.

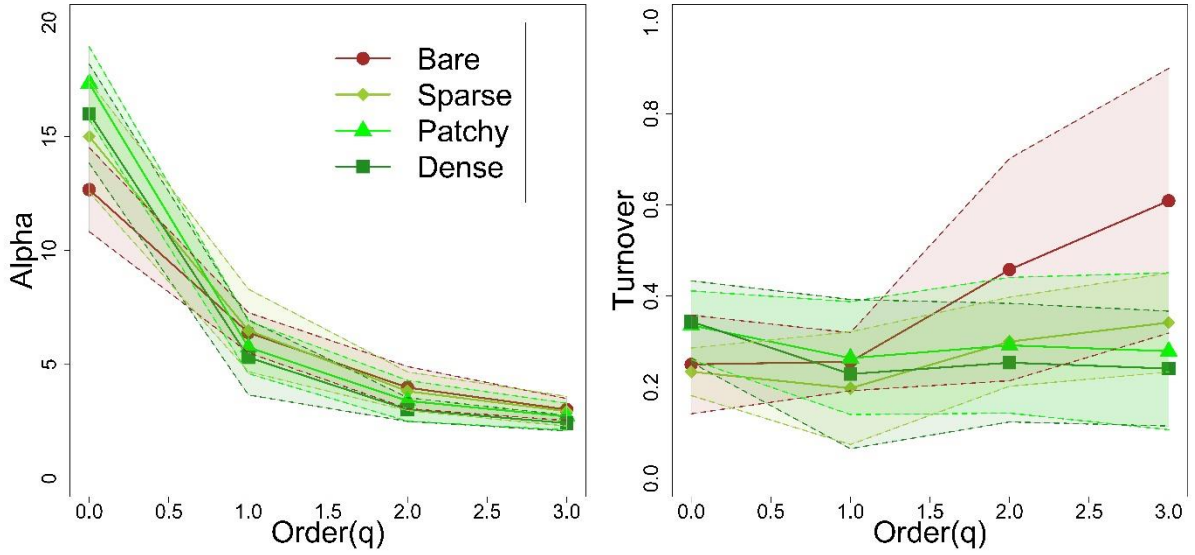

Figure 35: Hierarchical, group-wise diversity profiles for seagrass cover types. Estimates for alpha and turnover are plotted as a function of order q for replicates of region. Parameters for q define the down weighting of rare vs common species. As the value of q increases, the effective weight is assigned to the most common and abundant species. Thus, as q increases, the influence of rare species is suppressed. Values of q also represent orders of diversity where q=0 corresponds to the

estimates of true diversity (weighted harmonic mean), q=1 to the Shannon index (weighted geometric mean) and q=2 to the Simpson index (weighted arithmetic mean).

4.4 Discussion

Throughout the South Island, faunal communities associated with *Z. muelleri* habitat displayed significant variations in composition and abundance. Lateral patterns of diversity followed a southern trend in which diversity was highest in the south and lowest in the north. Regions were found to share a number of species, however, northern and southern sites differed the most in terms of species composition. In contrast, species composition for central sites shared similarities with both northern, but showed higher similarities to the north in terms of overall abundance. Species composition was also found to vary significantly within regions, with diversity and abundance highest in isolated sites. In contrasts, species composition and abundance for sites close to large-scale urban development were very different and showed signs of biotic homogenization

Seagrasses over space and time are subject to high levels of fine-scale variation in species composition (Aylesworth *et al.* 2017, Boström *et al.* 2006, Whippo *et al.* 2018). Spatial patterns of biodiversity in seagrass system constitute substantial parallel component of communities over latitude. Though, like most research on seagrass, density and diversity of various taxonomic groups have exhibited inconsistent latitudinal patterns (Virnstien et al 1984). Conventional theories on latitudinal patterns of biodiversity have proposed the concept that diversity increases towards the equator, attributed to the gradient of decreasing richness from tropical to non-tropical areas (Willig *et al.* 2003, Virnstien et al 1984). Nonetheless, as seen in this study, numerous exceptions of this pattern have been documented seen in nature. Seagrass-associated faunal communities sampled followed the opposite trend where diversity was highest in the south, and lowest in the north. Despite sharing several species, epifaunal composition between northern and southern communities varied greatly.

Out of numerous processes, perhaps the most recognised fundamental driver of latitudinal gradients is temperature, which influences mechanisms of metabolism, demography, and evolutionary processes (Reynolds *et al.* 2017, Schluter 2016). Nevertheless, increases in temperature have also been associated with elevated predation pressure, a considerable driver of community assembly in seagrass systems (Moles and Ollerton 2016, Reynolds *et al.* 2018). As suggested by this study, lateral distributions of species in *Z. muelleri* habitats in the south island may be driven more by factors such as temperature and predation pressure. When examining specie's contribution to proportional abundances, it is apparent that communities in the central and northern regions are heavily influenced by several crustacean species that occur regularly in highly abundant populations. The lack of an abundant crustacean community in the south may be attributable to seasonal differences as sampling

occurred over the colder months, as many species exhibit latitudinal variation that corresponds with reproduction (Littles *et al.* 2016, Wu *et al.* 2020).

More evidence of variation seen in seagrass associated communities is illustrated by the presence of clingfish at Delaware bay in the North. Although this bay has substantial seagrass habitat, the only fish caught were a species of clingfish, which was unusual as every other site sampled were characterized by large populations of juvenile flounder. Considering the condition of the bay leads me to believe that variations in fish community may be influenced by circumstances of landscape and local hydrology. For example, Delaware Bay is a well flushed, tidal lagoon situated as the bottom of a steep catchment and is characterized by a broad, shallow configuration and expansive tidal ranges (Gillespie 2008, Stevens and Robertson 2017, Šunde *et al.* 2017). During high extreme tides, the estuary fills to more than 5,300,000 m3 of brackish water, draining to as little as 23,000 m3 during extreme low tides leaving wide spaces of mudflat present with a single fast-flowing subtidal channel that drains into Tasman bay (Gillespie 2008, Stevens and Robertson 2017). Sampling this channel proved to be difficult on account of the fast-flowing current, which suggests that permanently residing species may require adaptations to avoid being washed out to sea. Clingfish, for example, have adapted modified pelvic fins that form a sucking disc, allowing firm attachment to various surfaces, hence are often affiliated with areas that are exposed to strong wave and current actions (Briggs 1995, Ebeling *et al.* 1970).

Throughout the past century, anthropogenic disturbances have led to drastic biotic homogenization of ecological communities. Biotic homogenization (or the loss of β diversity), the process by which two communities that are ecologically unique become progressively similar over time, has become a well-known trademark of the Anthropocene (Iacarella et al 2018). Due to their position in the catchment, coastal and transitional zones where seagrasses reside are particularly susceptible to anthropogenic pollutants (Short and Neckles 1999, Thomson *et al.* 2015). Across sites, there appears to be a similar trend where seagrass faunal communities were notable homogenised in areas that are exposed to a high degree of human activity. Seagrass beds in estuaries close to large scale urban development, such as the Avon Heathcote and Nelson haven, displayed significantly reduced numbers of species and abundance, irrespective of seagrass cover. Human population size and extensive land use have typically been used as a large-scale predictor of ecological impact in coastal habitats. (Drew *et al.*, 2015, Halpern *et al.*, 2008, Iacarella *et al.*, 2018).

One particularly interesting finding while sampling was the condition of seagrass meadows throughout the Papanui inlet along the south side of the Otago Peninsular While sampling the Papanui inlet almost all subtidal patches were dead, dying or completely covered by thick mats of Ulva lactuca (sea lettuce). This meant that sampling was restricted only to a small section of intertidal shore were living seagrass was present, despite intertidal patches being of questionable health. This came as a

surprise for several reasons. Firstly, this large amount of algae was likely recent, as algae has not been observed in Papanui inlet in either summer or winter (Mills and Berkenbusch, 2009). An algal bloom during the winter is surprising as alae usually thrives during the warmer months, especially as Papanui inlet is not near large scale urban development., unlike Portobello Bay. This large amount of algae could be explained by nutrient runoff from the surrounding agricultural land, a fact which has previously been attributed to seagrass die off (Orth et al 2006, Whippo et al, 2018). The presence of this bloom during the winter is particularly concerning, as a combination of nutrient runoff and warmer summer temperatures could result in the entire exclusion of seagrass from this bay, as has been found elsewhere. Papanui inlet is an expansive mudflat that extends roughly 4km before reaching the ocean, hence section sampled reflects a poor description of community composition. The vastness in spatial variation for seagrass associated species is attributable to the fluctuating environment where seagrass occur. By only capturing a small section of upper intertidal zone, which is typically the least inhabited region, we heavily lack evidence to make assumptions about this habitat as an entirety. Furthermore, individuals may also utilise the presence of ulva for refugia and resources thus, we cannot say that fauna in this bay is experiencing decline. Nonetheless, continuous algal growth to such a degree would have eventual and dire consequences for seagrass associated communities. One point for future concern is the potential for these blooms to increase throughout the warmer months, to the point where seagrass will be completely displaced.

One theory as to why this site experienced the bloom it did, when it did may have more to do with the physiology of the estuary, in conjunction with nutrient inputs from the surrounding lands. Papanui inlet is surrounded by agricultural land, with several large freshwater sources that drain into the inlet. As the bay fills and flushes very slowly, pollutants and excess nutrients are more likely to settle and accumulate rather than being washed away. It also became clear that the narrow gravel road that edges the inlet shore experienced moderate amounts of heavy vehicle traffic for an open-air quarry located near the upper intertidal shore. Due to the inlets size the upper tidal area where seagrasses were alive, and present is left exposed for considerable portions of time. Hence, it is possible that the reason these beds were still alive may rest on the physiological thresholds of sea lettuce to desiccation stress being much less than that of seagrass.

Major recognized anthropogenic threats for seagrasses and their associated communities include mechanical damage of beds through activities such as land reclamation, coastal construction, dredging in conjunction with the release of pollutants from (Erftemeijer *et al.* 2006, Haynes *et al.* 2000, Hemminga and Duarte 2000). Long term effects of seagrass survival begin to arise when activity changes local hydrodynamic characteristics such as current velocity and direction as well as depth profile (Ingram and Dawson 2001, Turner and Schwarz 2006). For example, loss of *Z. muelleri* in Stanley bay within the Auckland harbour, New Zealand has been attributed to the formation of a tidal stream after the construction of tide deflectors (Dromgoole and Foster 1983). Moreover, terrestrial

water ways transport run-off from agricultural and industrial land that if often laden with heavy metals, industrial chemicals, pesticides, plastics, and excess nutrients from unmanaged effluent and fertilisers (Brodie et al 2012, Gladstone-Gallagher *et al.* 2018). Increases in nutrient loads promotes problematic growth of phytoplankton, epiphytic algae, free-floating algae, and bottom-living algae which reduces light in the water column and induces eutrophication (Inglis 2003, Turner and Schwarz 2006, Valiela *et al.* 1997, Walker 2003). Furthermore, excessive epiphyte cover impedes seagrass leaf performance by disrupting gas and nutrient diffusion processes which leads to severe basification and CO2 depletion (Brodersen *et al.* 2015, Brodersen *et al.* 2020, Walker 2003). Negative impacts on seagrass habitat associated with epiphytic and macro algae blooms have been documented in numerous locations including parts of Japan, Australia, California and Europe (Han *et al.* 2016, Sugimoto *et al.* 2007). As indicated by this study, macro algal blooms have the potential to radically alter *Z. muelleri* ecosystems and associated fauna. Out of all sites, Papanui inlet was the only site with problematic algal growth and is also the site with the least diverse and abundant community. While this may be a consequence of sample location, we can hypothesize that the exclusion of seagrass throughout this area will express similar communities to those seen in the intertidal zones.

Issues such as these can be further exacerbated by major terrestrial disruptions such as fires, landslides, or forestry and land clearance that raise rates of erosion, resulting in higher sediment loads that can overwhelm the system (Haynes *et al.* 2000, Orth *et al.* 2006, Quiros *et al.* 2017, Serrano *et al.* 2016, Walker 2003). The combined effect of chronic increases in dissolved nutrients and suspended sediments causes significant reductions in light penetration which directly impacts seagrass growth and is considered to be the most ubiquitous and pervasive threat to the survival seagrass worldwide (Green and short 2003, Grech *et al.* 2012, Hemminga and Duarte 2000, Turner and Schwarz 2006, Unsworth *et al.* 2018, Walker 2003). The extent of sediment and nutrient loading many of New Zealand seagrass communities remains largely unknown, nevertheless, prolonged increases in both is expected to cause long-term and/or irreversible impacts (Turner and Schwarz 2006). Recreational activities such as boating and fishing also cause localised physical damage through trampling by cars and people and scouring by boat propellers, anchors, boat groundings and mooring damage (Spalding *et al.* 2003, Turner and Schwarz 2006). This often leads to habitat fragmentation which can alter assembly structure by reducing habitat area and connectivity and increasing predation rates on patch inhabitants (Mills and Berkenbusch 2009). As our results show, across all sites, patchy and dense habitat types supported more diverse and abundant communities compared to sparse and bare patches. Furthermore, sites with expansive seagrass habitat that consist of numerous microhabitats were also found to have high diverse and abundant communities, thus, supports the theory that seagrass with high structural complexity are more valuable habitat for a broad range of fish and invertebrate species.

The present study reveals strong evidence that proximately to large-scale human development may not be enough to infer the state of faunal communities in *Z. muelleri* habitats. Factors such as

surrounding landscape, topography and local hydrology also appear to play a large role in determining faunal compositions and should be taken into big consideration when inferring conservation actions. For example, as seen in Papanui inlet, bays surrounded by agricultural land are subject to high quantities of runoff which can disrupt sediment and nutrient balances throughout the ecosystem. Furthermore, the use of Z. muelleri by most species is made redundant when the surrounding habitat is under enough human-induced stress. This suggests that the presence of seagrass along is not enough to assume its biodiversity value.

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Final Discussion

Conducting ecological studies to establish a framework of spatial distributions, in addition with long-term monitoring programs are essential components of any effort to effectively conserve *Z. muelleri* and their associated biota in New Zealand. Across the South Island, *Z. muelleri* habitats displays vast differences in local abiotic conditions, species composition, and relative abundance. From single sampling events, it became evident that sites experiencing similar degrees of human interactions such as Nelson Haven, and Papanui inlet have significantly reduced diversities and abundances. In contrast, sites in more isolated areas displayed highly diverse and abundant communities. Despite regional variation in between sites, species composition and abundance for Duvauchelle and Papanui inlet were very similar. Further investigation into the temporal dynamics of these systems is required for an adequate understanding of how these systems are subject to change over time. As seen in Duvauchelle bay, epiphytic algae appeared to play a part in reducing overall abundance and diversity during the summer, a trend that was also detected at Papanui inlet in Otago.

The unusual timing of an extensive algal bloom also raises the concern of how this system will cope when conditions change in such a way that favours rapid algal growth. For example, flocks of grazing aquatic birds such as geese or swans often cause physical damage to seagrass beds, yet, the influence of invasive migratory waterfowl on the local seagrass nutrient loading rates remains understudied. Migratory waterfowl are important components in the transfer of terrestrial nutrients however, dense seasonal aggregations have been accredited to habitat loss and degradation of native wetland vegetation (Post *et al.* 1998). For example, large populations of migratory geese wintering at Bosque del Apache NWR in southern New Mexico resulted in a significant redistribution of nutrient loadings and poor water quality across the landscape (Post *et al.* 1998).

Despite that Duvauchelle bay and Papanui inlet were plagued with different algal species, we can expect that overall, increases in nutrient load and temperature are cause for concern relative to algal growth in *Z. muelleri* habitats. Moreover, seagrass ecosystems that thus far experience high nutrient loads from urban and agricultural runoff are at particular risk of eutrophication relative to estuarine physiology and hydrology. For example, in large flat landscapes with low drainage, and poorly flushed (e.g Papanui inlet), harmful materials are less likely to be washed away and will accumulate within the system. *Z. muelleri* habitats that are already experiencing abnormal or disruptive conditions may lack the ability to re-establish in the aftermath of macroalgal blooms (Olyarnik *et al.* 2012). In contrast, heathy systems that are not consistently subject to additional stressors, such as those in Duvauchelle bay will have the capacity to bounce, with respect to landscape (Evans *et al.* 2018, Tan *et al.* 2020, Short and Wyllie-Echeverria 2009). For example, despite having a flat topography, Duvauchelle Bay is a well flushed and mixed tidal lagoon in which much of the water leaves on the outgoing tide (Hume and Herndendorf 1988).

Implications for conservation and management

Seagrass habitats with high structural complexity can support a broader range of fish and invertebrate biota, thus, are recognized as more valuable for ecosystem services (McClosket and Unsworth 2015). Compared to unvegetated areas, *Z. muelleri* meadows contained a striking rich composition of fish and invertebrate species (Morrison *et al.* 2014, Orth *et al.* 1984). Nevertheless, as shown by the present study, *Z. muelleri* beds nestled within urban development have significantly reduced faunal communities, despite having considerable cover and habitat complexity. Thus, the presence of seagrass alone is not sufficient to infer faunal community health. This reinforces that effective management strategies of diverse faunal communities should focus on providing a stable and complex habitat with the adaptive potential to address the risks of human interference and climate change (Broadhurst *et al.* 2008, Jordan *et al.* 2019, Tan *et al.* 2020). Traditional approaches for restoration

have advocated mixed provenancing in which seagrass seeds from multiple sources are applied to a struggling ecosystem to aid in broadening the genetic basis of re-established seagrass populations (Broadhurst *et al.* 2008). Concerns surrounding this method involve non-local sources outperforming original populations under future climates, however, this does not pose the same threat in New Zealand as seagrasses are characterized by *Z. muelleri* (Tan *et al.* 2020). Areas such as Papanui inlet may particularly benefit from such strategies.

To date, there have been numerous restoration trials that have shown considerable success over several years however, the challenge remains to translate the small-scale and short-term into largescale restoration programs with consistent and long-term monitoring (Tan *et al.* 2020). Even at small scales, seagrass restoration is labour-intensive and expensive thus, upscaling into multi-year, funded restoration programs require the involvement of local communities, industry partners, local iwi, non-governmental organizations, and government agencies (Tan *et al.* 2020, van Katwijk *et al.* 2016). This is where using pipefish as a flagship species could come in handy to gain public attention and infer management decisions. For example, if a particular seagrass habitat is characterized by pipefish, we can assume that this ecosystem is of good health and high biodiversity value. Therefore, these sites should work on management plans that focus on maintaining biodiversity, whereas sites subject to heavy human influence should focus on maintaining ecosystem services.Given the highly variable nature seen in *Z. muelleri* ecosystems, restoration programs must take a holistic approach that focuses on landscape and hydrology to be effective. For example, local characteristics surrounding the nature of seagrasses such as shoot density and biomass, meadow size, abiotic conditions, seasonality, and light regulation in combination create specific ecological conditions that influence the direction and magnitude of observed faunal responses (Alsaffar *et al.* 2020). Furthermore, regular ecological monitoring programs are required to identify drivers of seagrass loss, in addition to establishing potential restoration locations and effectively determine the success of restoration projects (Duffy *et al.* 2019). The SeaSketch project put forth by the Department of Conservation as a national scale seagrass inventory is a promising start, however, this project is still lacking data for many unmapped locations (Anderson *et al.* 2017)

Supplementary to seagrass restoration, ecological engineering should be applied to elucidate conditions where settlement and re-established are promoted (Tan *et al.* 2020). As defined by Mitsch (2012), ecological engineering aims to generate sustainable ecosystems through integrated management that benefits both human society and the natural environment. Preliminary approaches for coastal ecosystems focus on replacing built infrastructure with restored mangroves or salt marshes or altering said infrastructure to promote settlement of native marine organisms by adding structural complexity (Dafforn, 2017, Loke *et al.* 2017). Conditions that drive *Z. muelleri* growth are well established, and combined with an understanding of site-specific conditions will be beneficial when

generating designs for ecological engineering (Tan 2020, Turner and Schwars 2006). Further opportunities for areas where coastal development is imminent is to incorporate ecological engineering, for example, building breakwaters may enhance settlement of seagrass seeds by mimicking shallow embayment's (Tan *et al.* 2020). Such strategies must also incorporate seed and patch dynamics, specific to *Z. muelleri*, such as dispersal, seed survival, and meadow connectivity (Broadhurst *et al.* 2008).

As restoration efforts increase in magnitude, plans must be managed similarly as largescale infrastructure development of which researchers may not have the experience or skill sets. To improve the likelihood of success, programs should incorporate a collaborative design that allows clear communication between researchers, managers, and relevant community stakeholders. Thus, skills, experiences, and perspectives across a range of disciplines are made available (Zedler, 2007). In return, researchers have the opportunity to gain insight into local socio-economic and local environmental conditions from regional managers and local iwi. The involvement of the community is key to successful and enriching conservation (Lin, 2019, Peters *et al.* 2015). For example, seagrass transplantation trials in Whangarei and the Porirua Harbours, New Zealand, benefitted greatly from the involvement of community volunteers (Tan *et al.* 2020, NIWA, 2009). Not only do volunteers represent a valuable resource for restoration efforts, but also a voice of awareness that is easily digestible by the public.

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